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INFANTILE GENETIC AGRANULOCYTOSIS

(Agranulocytosis infantilis hereditaria)

A New Recessive Lethal Disease in Man

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ALMQVIST & WIKSELLS BOKTRYCKERI AB

To the Memory of my Father

EDVIN SVEN VILHELM KOSTMANN

1885–1948

Preface

This investigation was started in 1949 when I was director of the Division of Pediatrics at the Army Hospital in Boden. The officials of the county of Norrbotten and, especially, the administrative director of public health, Mr. K. G. Wiklund, have taken an active interest in my work and contributed a substantial grant. The cost of this project was also in part defrayed by the Swedish Medical Research Council and the foundation of Ernst and Hilma Fredga.

As any other investigation, this one could not have been completed without the cooperation of a number of different institutions and individual scientists. Among the latter, I want to mention especially H. Magnusson, M.D., late Director of the Sachs' Hospital for Children in Stockholm and Professor B. Vahlquist, M.D. Uppsala. The cooperation in the genetic analysis of the material by Jan A. Böök, M.D., Ph.D., Uppsala, has been invaluable.

N. G. Nordenson, M.D., Associate Professor of Hematology, and F. Wahlgren, M.D., Associate Professor of Pathology, both at the Southern Hospital in Stockholm, have greatly contributed by the analyses of the blood, bone marrow and histological specimens.

The help and encouragement received from all of them is gratefully acknowledged.

Contents

Introduction

In this work, a new disease, *infantile genetic agranulocytosis*, will be described. As a rule, the outcome of this disease is fatal within the first year of life. The children succumb to infections of various kinds, most commonly manifested as septic skin conditions. The affected children have been discovered in a certain district in Norrbotten, the northernmost province of Sweden (see Fig. 1). The disease appears to depend primarily on a single recessive gene difference.

Chapter I

A. Collection of Data

In March 1949, *a two months old girl* (case A9) was admitted to the Children's Hospital in Boden, one of Sweden's northernmost towns. *She displayed multiple abscesses in the skin*, otitis, mastoiditis, and high fever. *She had complete agranulocytosis.* With antibiotic therapy, the infection could be counteracted, and the child was kept alive for almost four months. During the whole course of her disease, almost complete agranulocytosis was present. *The mother had earlier given birth to eight children; of these, four had died as infants, 26, 29, 30 and 32 days old, respectively. In the last few weeks of life, three of these four children had high fever, as well as furuncles and abscesses similar to those of the ninth child now in the hospital.* Two of these children died in the infirmary of Överkalix with the diagnosis of carbunculosis and pemphigus, respectively. The third child died at home, where it was under the observation of the district nurse. The fourth child also died at home, with the diagnosis of pneumonia. *The mother claimed that similar diseases had occurred among infants in her husband's family, and that all affected children had died.*

As it seemed probable already from the start of my inquiry that this disease was genetically determined, that it appeared in children, predominantly infants, and that it usually led to death, a preliminary investigation of the family of the father was undertaken. All infants who had died by the age of six months were registered as suspects of the disease.

In the last generation, 83 children had been born, divided between 18 families. Of these 83 children, 14 died before the age of six months. No further children had died by the age of one year. This corresponds to an infant mortality of 168.7 per thousand. All children of the last generation were born after 1930 within the area of the Överkalix church registry, and all except two had died within this area. The infant mortality in this area within the last 18 years had been 225 dead of 4035 born, i.e. 55.8 per thousand. *Thus, the infant mortality in the generation of the family concerned had been three times as high as in the municipality as a whole.*

None of the 14 children had died as newborn. If one does not include those that died during the first week of life, the following figures are obtained:

For the municipality:	152 dead in 4035 born	= 37.7 per thousand
In the family:	14 dead in 83 born	= 168.7 per thousand.

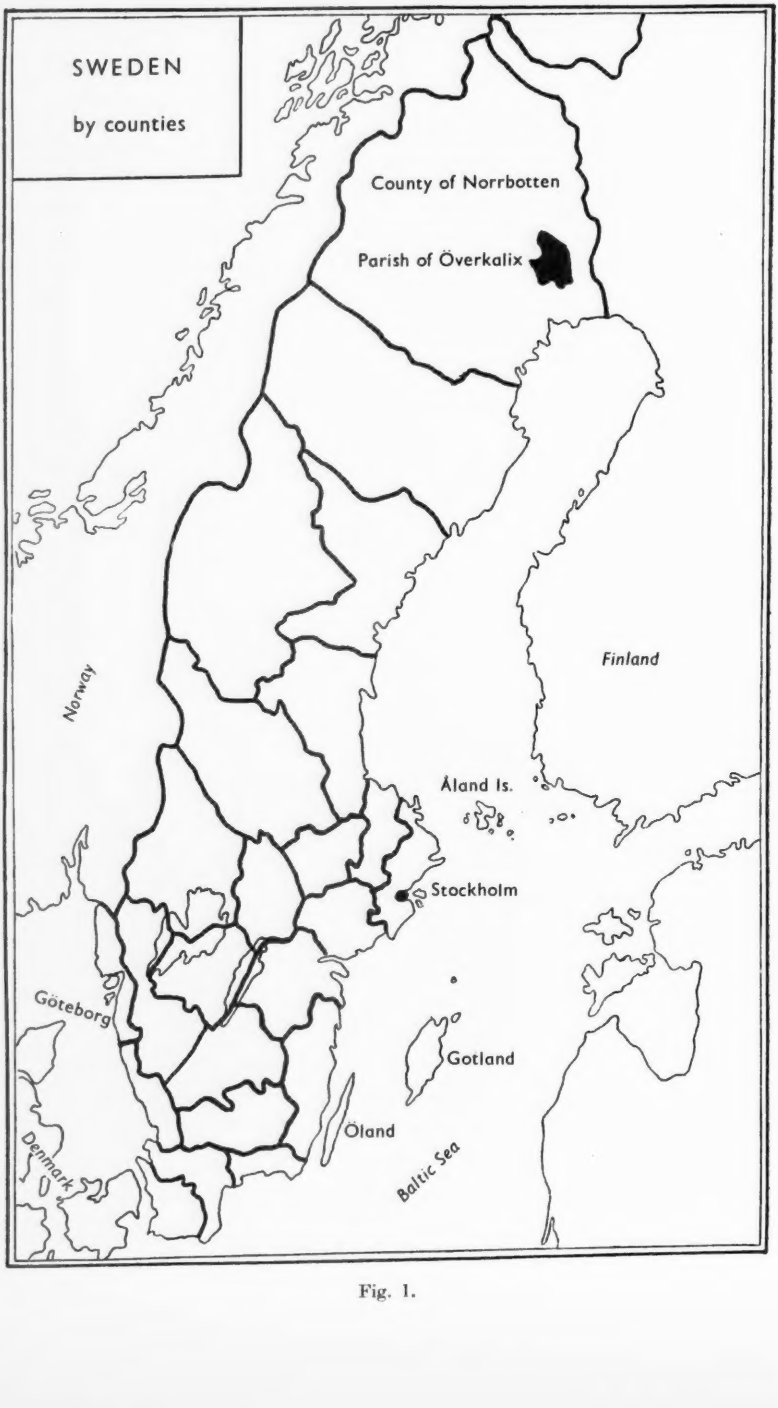

SWEDEN
by counties
County of Norrbotten
Parish of Överkalix
Norway
Finland
Åland Is.
Stockholm
Göteborg
Gotland
Öland
Denmark
Baltic Sea

Fig. 1.

The most obvious symptoms of the first case were furuncles, abscesses and skin infiltrations, with or without sores and fusions. If such signs had appeared in earlier, not-diagnosed cases, they ought to have been observed by the parents and others who had seen the children during the disease.

By questioning parents, district nurses, and doctors concerning the histories of the dead children, it could be established that eight of them had displayed symptoms similar to those of the first case. All of them had had furuncles, abscesses, phlegmons and high fever.

One of the cases discovered in this manner (case D1), had been treated in a hospital (Gällivare) and died there. According to the hospital record, the cause of death was agranulocytosis. Blood slides and pathologic-anatomic preparations had been kept, and an examination of these revealed changes identical to those of the first observed case.

A continued, systematic survey was made in the church records of the claimed causes of death of all infants that had died in the municipality during the last eighteen years. In cases where suspicious causes of death were present, more detailed information concerning the disease and manner of death was obtained from parents, physicians and nurses. For the same purpose, inquiries concerning individuals with suspicious diseases and causes of death were made in all neighboring hospitals. During the course of this investigation, a few additional individuals with this disease were discovered. The one that was most valuable for the investigation (case R4) had been referred to the hospital in Boden by a physician—Dr. *Bjarke*, to whom I am greatly indebted—who had recognized the disease through my first publication (Kostmann 1950).

All now-living families affected by the investigation have been visited by me, and I have personally recorded all information. The catamnestic statements were given by parents of the deceased children, and complementary information was provided by physicians, district nurses and midwives.

The genealogic data have been collected through the parish church registers. These investigations were partly performed by me, and partly by my assistant. Investigations were also made in the province archives in Härnösand, where the old registers are kept. During this work, a total of some 700 individuals has been investigated.

All parents and siblings of those children who had been judged to suffer from the disease or had died from it have been examined. In addition to a general physical examination, a blood examination was included, consisting of a hemoglobin determination, a white blood cell count, and a differential count. In one family (family A), an examination of sternal punctates of both parents has been undertaken. In two families (families

D and F), the same examination has been performed on the mothers. All these individuals displayed normal findings and were also generally healthy.

It should be added that all unaffected members of the families, adults as well as children, were physically and mentally well-developed individuals. All the families lived under fairly good economic conditions and had good, clean homes. Most of them could, without difficulty, be classified as belonging to the ordinary Nordic type. Only a few ancestors of Finnish or Lappish derivation were disclosed.

B. Classification of the Affected Individuals

With regard to the completeness of the examination and the degree of diagnostic certainty, the affected children have been classified into two groups:

Group I. *Six children* who have been observed in hospitals and who have been subjected to more detailed pathologic studies.

Group II. *Eight children* who, on account of catamnestic and genealogical information, have been judged as affected with the same disease entity. All have died as infants in septic diseases and have had furuncles, abscesses, and phlegmons.

The relationship of the different individuals are shown in the pedigrees (Fig. 15, p. 67). The sibships have been noted by capital letters and individuals by Arabic figures according to birth rank.

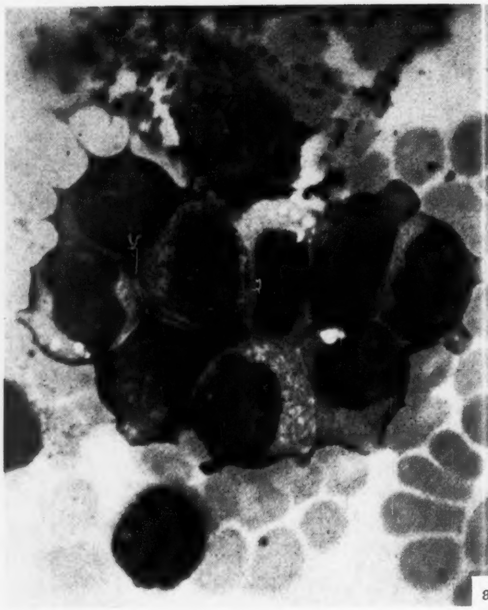
Chapter II

The Affected Children of Group I

Case A9

Girl. Born January 7, 1949. Died June 26, 1949. Normal delivery. Weight at birth, 3000 g. Vaccinated against tuberculosis. She was breast-fed for $1\frac{1}{2}$ months. At the age of three weeks, she fell ill with hoarseness and fever; was treated with steam and sulfa drugs, 0.125 g. $\times$ 5 for three days, and recovered. At the age of four weeks, she had a right-sided otitis. She was taken to the local infirmary, and treated with penicillin. At the age of $1\frac{1}{2}$ months, she was transferred to the otologic department of the hospital in Boden. She had mastoiditis on the right side and was highly febrile. The mastoid cell system was large for her age, and the posterior wall of the auditory canal was almost completely destroyed. The cell system was entirely removed, and the wound cavity was filled with penicillin. The wound was sewn primarily, and a penicillin tamponade was placed in the auditory canal. She became free of fever and the recovery appeared normal. Three weeks after the operation, she got high fever, multiple boils (furuncles) and a complete agranulocytosis. She was transferred to the children's hospital, now 2 months and 10 days old.

Her general condition was strongly affected. She was highly febrile and pale-cyanotic. There were eight elevated, strongly reddish, furuncle-like eruptions the size of hazelnuts on the lower part of the abdomen and on the thighs. Nearly all had centrally a bluish-grey miscoloring about the size of a pea with blister formation or a pasty, necrotic sore of the same size (Fig. 2, p. 12). The superficial lymph nodes, liver and spleen could not be felt. The blood examinations have been summarized in Fig. 3 and Table I. Cultures from the blisters yielded growths of a pathogenic strain of Staphylococcus aureus, streptomycin sensitive but penicillin and sulfa resistent. A blood culture was negative. Wassermann reaction in blood negative. Mantoux 1 mg negative. She belonged to blood group A_1MNS Rh_1 (father: A_1MP Rh_1, mother: OMN Rh_1). Skeletal X-ray was negative. She was treated with streptomycin and blood transfusions. After ten days, her temperature returned to normal. Her general condition had improved considerably, and the furuncle-like skin changes had assumed a brown or blue-grey color. The central parts had been sloughed off, and the sores had cleaned up and were partly healed.



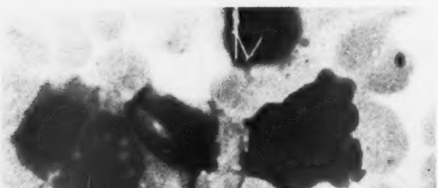


Fig. 2. Case A9. *a*, *b* and *c*, microphotographs of bone marrow smear. Vacuolisation, atypical nuclei and predominance of myelocytic cells.

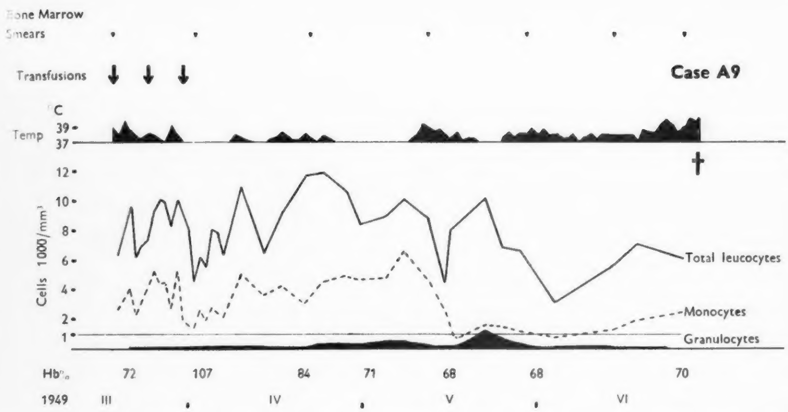


Fig. 3. Case A9. Graphical representation of the blood values during the course of illness.

During the next few weeks, her general condition was relatively unaffected despite sub-febrility and signs of infection of the upper respiratory tract. However, the skin color remained pale and tonus and turgor were reduced.

At the age of four months, new furuncles appeared on the abdomen, back, thighs and gluteal regions. The temperature rose again to over 39°C. Her general condition became worse. Enlargement of the liver, spleen and lymph nodes could not be observed at this time, either. After renewed streptomycin treatment for ten days, the temperature dropped again. The local skin processes improved.

The operation wound behind the right ear had healed. The cartilaginous part of the ear channel had sequestered and suppuration continued. An easily-bleeding polypus had formed in the ear channel.

At the age of 4½ months, the child was transferred to the Sach's Hospital for Children in Stockholm.

During the continued course of the illness, the fever was constantly about 38°C. Occasionally, new furuncles appeared. Incisions of melted, ripened furuncles and abscesses were made. Moderate quantities of hemorrhagic pus were liberated. In cultures from this pus, there still grew pure strains of Staphylococcus aureus. During the last weeks of her life, the liver could be felt. In the final stage, it reached half way between the xiphoid process and the level of the navel in the median line. It had a relatively solid border. Some superficial lymph nodes of the size of grains could be felt in the inguinal and axillary regions. On the wall of the chest along the left axillary line, a solid gland the size of a bean appeared, which was excised for histologic examination.

The general condition of the child became gradually worse with eruptions of new furuncles, inflammatory infiltrations, violent stomatomycosis and high fever. She finally died at the age of 5 months and 22 days.

Special Examinations

Blood examinations have been summarized in Table I (p. 15) and Fig. 3 (p. 13).

Bone Marrow Examinations (N. G. Nordenson, M.D.)

March 17, 1949: Sternal punctate: Cell-rich preparation. In myelopoiesis, a block in the maturation process after promyelocytes was observed. Signs of toxic maturation disturbance were noted. The eosinophilic cells were slightly increased. Erythropoiesis was moderately hyperplastic with normal maturation. The megakaryocytes were normal.

Diagnosis: Granulocytopenia. Grave changes. Nothing definitely specific in the picture.

April 1, 1949: Sternal punctate: Scanty cell preparation. Myelopoiesis was only represented by a few myeloblasts. Erythropoiesis was sparse. The megakaryocytes were normal. The reticulum was slightly hyperplastic.

Diagnosis: Agranulocytosis. Same picture as in the previous examination.

April 21, 1949: Sternal punctate: Relatively cell-rich preparation. Myelopoiesis was strongly inhibited with a block after myeloblasts-promyelocytes. Erythropoiesis was largely normal. The megakaryocytes were very rare. The reticulum was greatly hyperplastic.

Diagnosis: Granulocytopenia. The cells were now more immature with an increase in myeloblasts, of which many had atypical forms of the paramyeloblast type. The findings suggested an acute leukemia.

May 11, 1949: Sternal punctate: Scanty cell preparation. In principal, the same changes as in previous punctures were noted. The picture was dominated by reticular and mononuclear cells which partly resembled micro-myeloblasts.

Diagnosis: Granulopenia with some suspicion of acute leukemia.

May 28, 1949: Sternal punctate: Cell-rich preparation. In myelopoiesis, a maturation inhibition was noted with a block in the maturation process after myelocytes. The findings suggested toxic maturation disturbances. In erythropoiesis, signs of maturation disturbance (polychromasia) were observed. The megakaryocytes were rare. The reticulum was strongly hyperplastic.

Diagnosis: Strongly reactive bone marrow. Granulopenia. Systemic disease?

June 13 1949: Sternal punctate: Cell-rich preparation. In myelopoiesis, the strong maturation inhibition with block in the maturation process after myelocytes still dominated. Monocytoid reticular cells were rather numerous. In erythropoiesis, a strong maturation disturbance was noted. The megakaryocytes were normal. The reticulum was strongly hyperplastic with an increase in lymphoid cells.

Diagnosis: Granulopenia. Systemic disease? Worse condition since the previous examination.

Table I

Case A9. Blood examinations.

Date	Hemoglobin %	Red blood cells million pr mm³	White blood cells pr mm³	Stab cells %	Segmented neutrophils %	Eosinophils %	Basophils %	Lymphocytes %	Monocytes %	Unspecified cells %	Platelets pr mm³	Sedimentation rate
18/3	72	4.22	6,400	—	—	—	—	57.5	41.5	1		56
20/3			9,600	—	—	0.5	—	55	43.5	1		
21/3			6,200	—	—	5.5	—	56.5	37.5	0.5		
22/3			7,000	—	—	2	—	52	45	1		
23/3	75	4.02	7,400	—	—	0.5	—	43.5	56	—		33
24/3			9,200	—	0.5	1	—	38.5	57	3		
25/3			10,100	—	—	1	—	54	44	1		
26/3			10,000	—	—	—	—	49	45	6		
27/3			8,400	—	1	1	—	60	33	5		
28/3			10,000	—	0.5	0.5	—	43	54	2		
29/3	84	4.47	3,700	—	1	1	1	47	49	1		38
30/3	110		8,100	—	—	—	—	60	39	1		
31/3			6,500	—	—	1	1	65	32	1		
1/4			4,600	—	—	—	—	67	31	2		
2/4	107		6,200	—	1	2	—	53	44	—	260,000	
3/4			5,600	—	1	1	1	62	35	—		
4/4			8,100	—	—	3	1	60	34	1		
5/4			7,800	—	—	2	1	64	31	1		16
6/4	97		6,400	—	1	2	1	62	33	1		
9/4	100		11,000	—	—	—	—	52	47	1		
13/4	93	4.68	6,500	—	—	2	—	40	58	—		26
16/4			9,300	—	—	1	1	50	46	1		
20/4	84		11,700	—	—	1	0.5	71.5	26.5	0.5		52
23/4			11,900	—	—	3	—	57	38	1		
27/4	87		10,700	—	—	3	—	50	46	1		53
29/4											393,000	
30/4			8,500	—	1	3	—	40	55	1		
4/5	76	4.26	9,000	—	—	6	—	38	54	1		
7/5	98	4.90	10,100	—	—	5	—	35	60	—		
11/5	63	3.54	8,900	—	1	2	—	42	53	1		
14/5	68	3.90	4,500	—	—	1	—	61	34	1		
15/5			8,100									
21/5	72	3.64	10,200	1	12	—	—	84	3	—		
24/5	71	3.39	6,800	1	10	—	—	88	1	—		
7/5	75	3.48	6,600									61
3/6	73		3,100	—	5	—	—	70	25	—		68
3/6	72	2.10	5,600	—	—	—	—	78	22	—		60
7/6	70	3.20	7,000	—	1	1	—	70	28	—		
5/6	70	2.78	6,100	—	—	—	—	68	40	—		

23/3 Protrombin index 63 Bleeding time: 2 min 35 sec
2/4 „ „ 92 Clotting time: 3 min 30 sec
Bleeding time: 1 min 30 sec
9/4 „ „ 89 Clotting time: 9 min 55 sec

June 25 1949: Sternal punctate: Relatively scanty cell preparation. The picture was dominated by a strongly hyperplastic reticulum with monocytoid elements. The myelo-erythropoiesis was extremely sparse.

Diagnosis: Granulopenia. Systemic disease?

X-ray examination of heart and lungs. May 2, 1949: Heart of normal size. In the lungs, intensified striation in hilus medially towards the bases and parenchymal condensation basally behind the heart.
X-ray examination of skeleton. May 2, 12, 23 and June 22, 1949: No pathologic structural changes.
Ophthalmological examinations. June 22, 1949: Sclerae somewhat bluish, but not definitely pathologic. Otherwise normal conditions.
Electrocardiogram. May 8, 1949: Normal.
Lumbar punctate. April 22, 1949: Normal conditions. No growth upon culturing the spinal fluid.

Bacteriologic Examinations

Blood culture, March 17, 1949: No growth. Material from pus blister, March 17, 1949: Growth of Gram-positive cocci (Staphylococci). March 19, 1949: Rich growth of pathogenic strain of Staphylococcus aureus. Penicillin and sulfa resistent. Streptomycin sensitive.

Throat culture, May 21, 1949: Growth of α-streptococci: Sulfa and streptomycin resistent. Penicillin sensitive. Staphylococcus aureus: Sulfa resistent. Penicillin and streptomycin sensitive. Coliform bacteria: Sulfa, penicillin and streptomycin resistent.

Nose culture, May 21, 1949: Growth of α-streptococci: Sulfa and penicillin sensitive. Streptomycin resistent. Staphylococcus albus: Sulfa resistent. Penicillin and streptomycin sensitive.

Secretion from the right ear, May 21, 1949: Growth of Bacillus pyocyaneus. Sulfa, penicillin and streptomycin resistent.

Secretion from necrotic sore in the scalp, May 21, 1949: Growth of α-streptococcus. Sulfa and streptomycin resistent. Penicillin sensitive. Staphylococcus aureus: Sulfa resistent. Penicillin and streptomycin sensitive.

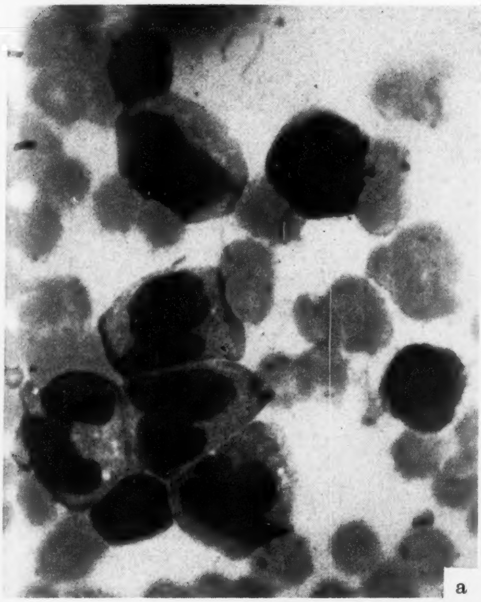
Secretion from necrotic sore on the back, May 21, 1949: Growth of Staphylococcus aureus: Sulfa resistent. Penicillin and streptomycin sensitive.

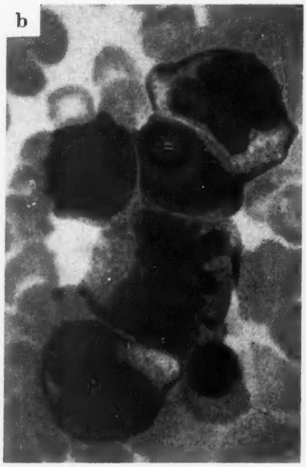
Pus from abscess, May 23, 1949: Growth of Staphylococcus aureus: Sulfa and penicillin resistent. Streptomycin sensitive.

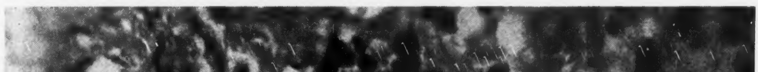
Pathologic-Anatomic Examinations **(Fredrik Wahlgren, M.D.)**

Histological examination of a *lymph node* from upper part of thorax (June 2, 1949): In the examined lymph node, which was the size of a coffee bean, the normal architecture was erased. This appeared due to very large growth centers consisting of pathological cells in the lymph follicles. The different follicles were separated only by narrow streaks of lymphatic tissue with lymphocytes and somewhat enlarged reticular cells. In addition, there were a few eosinophilic leucocytes in the tissue, but otherwise no pathological cell elements were seen. The vessels of the lymph node had markedly enlarged endothelial cells. The sinus appeared compressed and empty. The lymph node was inbedded in a thick capsule of cell-poor, dense connective tissue in which there were scattered lymphocytes as well as some large mononuclear cells, which, in their structure, most closely resembled the cells in the germinal centers.

Fig. 4. Case A9. *a* and *b*, Microphotographs of bone marrow smears. *c*, Bone marrow (autopsy specimen). Predominance of immature red cell forms.







Autopsy

June 29, 1949: A child of ordinary size for its age with extremely sparse, slack flesh and pale grey skin color. Weight 4600 g. On the left side of the skull were a few brownish-violet papules in the skin, scarcely the size of hemp-seeds. Over the left kidney region were two large, irregular, dirty-brown-violet skin infiltrations, partly covered by yellowish-brown, dried crusts.

The brain and its membranes showed no changes. The bones of the skull were also normal. The hypophysis was of normal size.

The thymus was strongly reduced in size, and consisted of a thin, reddish-grey disk.

The pericardium contained a small amount of clear fluid. The heart and the large vessels had no malformations or other changes. The heart muscle was a pale reddish-brown.

Both lungs were free in the pleurae, which did not contain any fluid. The lung parenchyma was fully air-carrying and gave up a small amount of foamy, clear fluid from the surface of incision. In the trachea and bronchi, the mucous membrane was pale with some slimy, foamy coating.

In the posterior mediastinum, the lymph nodes were, at most, the size of coffee beans with dark greyish-red incision surfaces.

In the throat and upper part of the esophagus, the mucous membrane was pale grey with a few greyish-yellow, grainy coverings of the size of a hemp seed. From the upper chest aperture down to cardia, the esophagus was strongly dilatated in a funnel-shaped manner. Its mucous membrane was changed into dirty-greyish-yellow, grainy masses.

There was no free fluid in the abdominal cavity. The mucous membrane of the stomach was pale grey and without changes. The mucous membrane of the small intestines was also normal. There was no enlargement in the lymphoid system. On the ileocecal valve, there was a small, dirty-greyish-brown, grainy coating. The mucous membrane of the colon was pale and normal. The liver was of normal size and consistency, and had a pale reddish-brown incision surface which did not show any obvious architecture. Gall passages, the pancreas, and the portal vein were normal. The spleen was of normal size and consistency and had a dark red, even incision surface. Weight: 10 g. The adrenals were of normal size. The cortex was thin and rich in lipoid material. The medulla was pale greyish-brown. The kidneys were of normal size. They were pale, but otherwise normal. The urinary passages were normal. The normal curvature of the spine was completely straightened out. The bone tissue was dark red in the vertebrae and ribs.

Histological Examinations

Within the examined parts of the *lungs*, the alveoli were mostly filled with air, but occasionally contained a small amount of serous fluid or red blood corpuscles. The septae of the alveoli were very wide and rich in cells, with the vessels rather heavily filled with blood. There was also an accumulation of lymphocytoid cells and an enlargement of the reticular cells. There was no sign of giant cells or necrosis. The bronchi occasionally contained a small amount of mucus. The mucous membrane, as well as the wall, showed no obvious changes. In the mucous membrane of the *esophagus*, there were many ulcerations covered with necrotic masses with large amounts of fungi (Oidium). Around the ulcerations, the wall showed rather pronounced inflammatory changes.

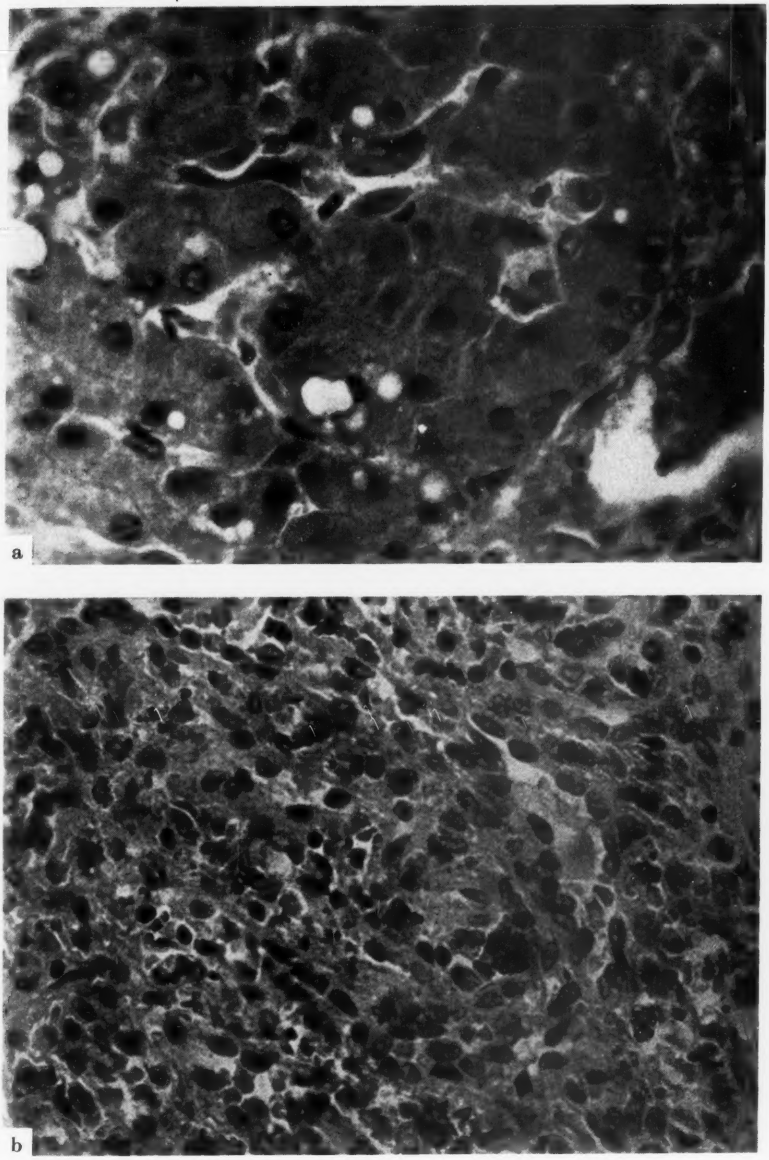


Fig. 5. Case A9. Autopsy specimens. *a*, Liver: Swelling of Kupfer cells and signs of fatty degeneration. *b*, Spleen: Swelling of sino-endothelial cells.

The *thyroid*, as well as the musculature of the neck, was histologically normal. In the *tonsils*, the lymphoid tissue was hyperplastic with enlarged reticular cells. There were no prominent germinal centers and no pathological cell elements were observed.

The follicles of the *spleen* (Fig. 5b, p. 19) were very small and lacked germinal centers. The pulp was devoid of blood and rather rich in cells. There was some enlargement of the sino-endothelial cells, but the pulp cells proper did not appear particularly enlarged. The pulp contained a very large amount of blackish-brown iron pigment. No pathological cell elements were observed.

In sections of the *small intestines*, an ulceration was found which, in some parts, penetrated to the outermost muscle layer. The base of the ulceration consisted of necrotic tissue which, at the bottom, changed into granulation tissue containing lymphocytes, plasma cells and fibroblasts. No giant cells were observed. The outer layers of the wall were edematously loosened and rather rich in inflammatory cells. In the serosa, fibroblast regeneration was observed.

The *lymph nodes of the mesentery* showed no gross changes. The tissue was rich in blood but it had, in general, a normal architecture. Possibly the lymph sinus contained a somewhat increased number of enlarged desquamated endothelial cells. Otherwise, no pathological cell elements were noted. The vessels and nerves of the mesentery appeared normal.

The acinous architecture of the *liver* (Fig 5a, p. 19) was rather distinct. The liver-parenchymal cells showed signs of fine-dropped fattification. The intra-acinous capillaries were collapsed and poor in blood. Quite a number of the Kupffer astral cells were enlarged. No signs of phagocytosis could be observed within them. No increase in periportal connective tissue was observed. Many Kupffer cells contained rather large amounts of brown iron pigment. Gall passages and vessels were normal. The *pancreas* did not show any noticeable histological changes.

In the *adrenals*, the architecture appeared normal. The tissue was rather rich in lipoid material. The medulla contained large amounts of blood in which there were also numerous phagocytes containing iron pigment.

The *bone marrow* (Fig. 4c p. 17) was very poor in cells and appeared to contain mainly early precursors of the red blood cells. There were very few cells belonging to the leucocyte series. Megakaryocytes appeared in approximately normal numbers. The reticular cells were distinctly enlarged.

Epicritic Summary

A girl fell ill when three weeks old with an infection of the upper respiratory tract, which was complicated by otitis and mastoiditis. The latter was radically operated, but showed a poor tendency to heal. Multiple furuncles and abscesses appeared in the skin. She got high fever and her general condition worsened. The peripheral blood showed agranulocytosis. A maturation block at the promyelocyte-myelocyte stage was found in the bone marrow. The continued course of the disease was, above all, characterized by recurring skin afflictions. These could be counteracted by different antibiotics, but recurred when the treatment was discontinued. Her general condition became gradually worse. Finally, she became cachectic and died at

the age of six months. During the whole course of the disease, she displayed a pronounced granulocytopenia or agranulocytosis.

The clinical history, the blood picture, the intravital studies of the bone marrow, and the results of the post-mortem histologic examination justify the diagnosis of agranulocytosis. No findings indicated that this agranulocytosis should have had an exogenous toxic or allergic origin.

Case D1

Girl. Born March 12, 1948. Died June 17, 1948. Normal delivery. Weight at birth, 2810 g. She was breast-fed for two months. At the age of three weeks, a bluish-red infiltration appeared on one popliteal region. The girl became fussy, cried a great deal, and ate poorly. At the age of one month, she was taken to the surgical ward in Gällivare Hospital. She was then highly febrile and generally affected. On the one buttock, on the right popliteal region, on the right shoulder, on the neck and behind the one ear were necrotic sores, and in the face and on the chest were some bluish-red infiltrations. She was treated with penicillin and became free of fever. The sores began to heal and she was discharged from the hospital. Two weeks later, an incision of a furuncle on the right popliteal region was made. After an additional three weeks, she got high fever again. She also vomited and had diarrhea. She was taken to the medical ward of the same hospital.

Status June 12, 1948: The general condition was considerably affected. She was pale and had toxicosis. No dyspnea. Fontanel normal. The larynx was considerably inflammed. A few rhonchi could be heard on the lungs. Liver, spleen and superficial lymph nodes could not be felt. She died June 17, 1948 when 3 months and 5 days old.

Special Examinations

Blood Examination, June 13, 1948: Hemoglobin 44 per cent; red blood cells 2.3 million per cu.mm.; white blood cells 800 per cu.mm. Differential count: myelocytes 14 per cent; metamyelocytes 49 per cent; lymphocytes 37 per cent (?). Thrombocytes 590,000 per cu.mm. Prothrombin index 72 per cent. SR 41 mm/1 hr. Blood groups: O Rh +. Mother: O Rh +. June 14, 1948: White blood cells 750 per cu.mm. Differential count: myelocytes 3 per cent; metamyelocytes 1 per cent; lymphocytes 96 per cent. June 15, 1948: White blood cells 1600 per cu.mm. June 16, 1948: White blood cells 1800 per cu.mm. June 17, 1948: It was noted in the records: The white blood corpuscles have increased somewhat in number, but not a single granulated cell can be seen.

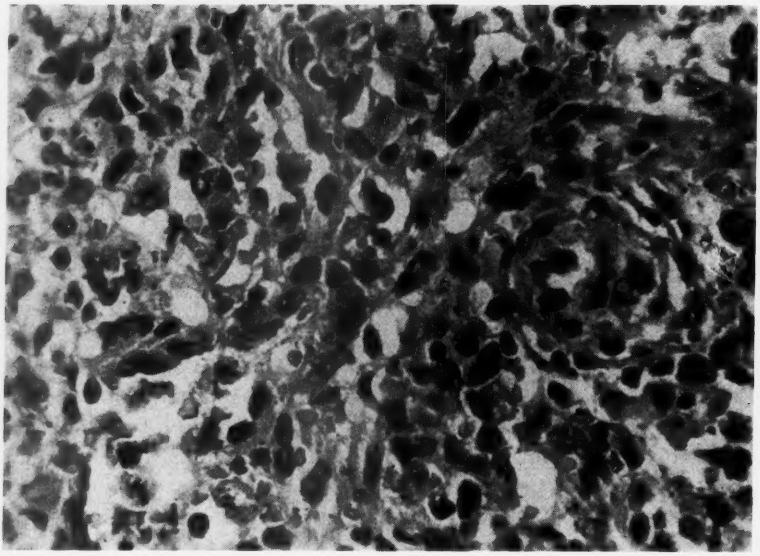


Fig. 6. Case D1. Autopsy specimen. Spleen: Swelling of sino-endothelial cells.

Autopsy

The heart was normal. The pericardium contained large amounts of blood-colored fluid. Fibrin deposits were found on the visceral pericardium. The lungs adhered to the pleura, and could only be freed from it with difficulty. The lungs felt as if they were penetrated by small infiltrations, probably bronchial pneumonias. There was no free fluid or fibrin deposits in the abdominal cavity. The intestines were meteoristic. The liver was pale, the spleen small and the kidneys normal. The renal pelvi contained a thin, pus-like fluid.

Histological Examination (Fredrik Wahlgren, M.D.)

The follicles of the *spleen* (Fig. 6) were small and lacked germinal centers. They showed neither sclerosis nor other regressive changes. The pulp was moderately rich in blood. The sinus contained relatively few red blood cells. The sino-endothelial cells appeared somewhat enlarged. The pulp was rather rich in cells, which were enlarged. In addition, it contained a few leucocytes, among which were also a few eosinophils. The pulp appeared to be richer than usual in collagen, but silver staining did not demonstrate any obvious increase of the reticulum. There were some local centers of destruction in the spleen tissue.

The acinous architecture of the *liver* (Fig. 7b) appeared well preserved. The liver parenchymal cells showed moderately pronounced large-dropped fattification. The intraacinous capillaries were moderately wide and contained little blood. The Kupffer astral cells were enlarged. The periportal connective tissue seemed somewhat more abundant than usual and contained a few mononuclear cells. Gall passages and vessels were normal.

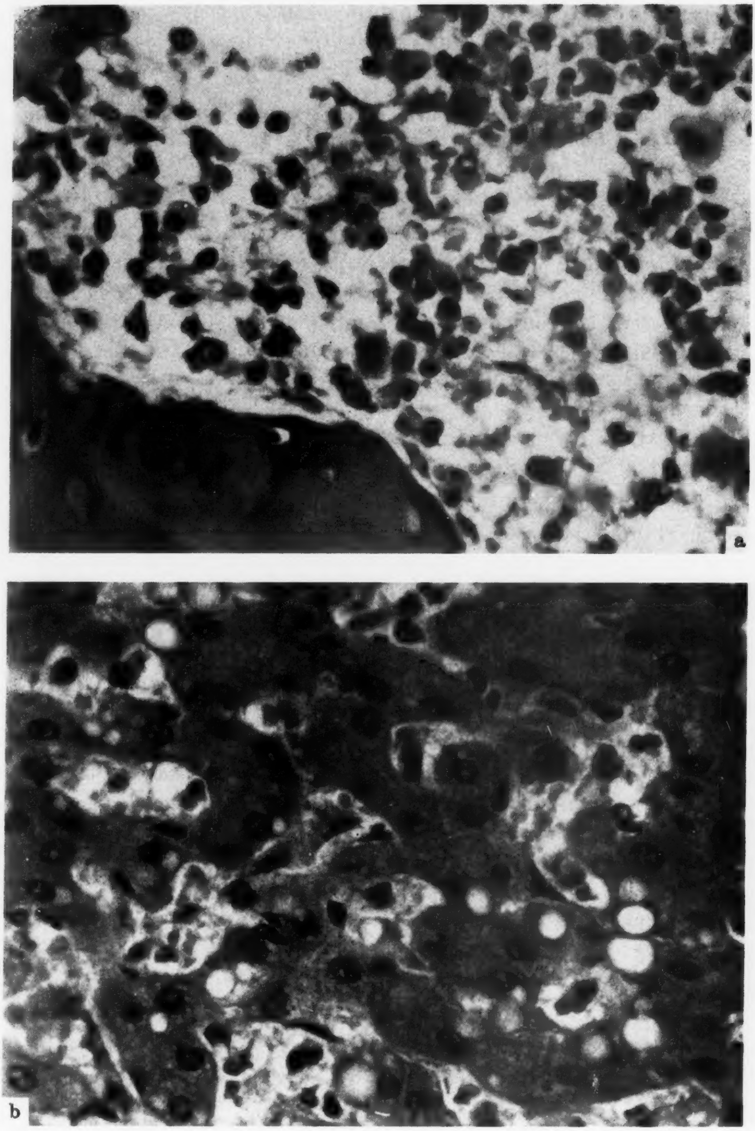


Fig. 7. Case D1. Autopsy specimens. *a*, Bone marrow: Poverty of cells and predominance of immature red cell forms. *b*, Liver: Swelling of Kupfer cells and signs of fatty degeneration.

The *bone tissue* of the ribs (Fig. 7a, p. 23) did not show any changes. The bone marrow was relatively poor in cells. It appeared to contain predominately precursors of erythrocytes. There were a few pathological cell elements which were interpreted as megaloblasts. The reticular cells were distinctly enlarged. The megakaryocytes were few in number and displayed pathological forms with peculiar, chromatin-rich nuclei that showed signs of disintegration.

Epicritic Summary

A girl fell ill at the age of three weeks with a phlegmonous infiltration on the one popliteal region. When taken to the hospital at the age of one month, she was highly febrile and had multiple septic infiltrations and necrotic sores of the skin. She was treated with penicillin and incisions. The septic skin afflictions recurred several times, and the child was treated ambulantly with incisions and penicillin. At the age of three months, she was again taken to the hospital. She was then highly febrile and moribund. The peripheral blood showed leucopenia, agranulocytosis and anemia. She died after a few days at the age of 3 months.

The clinical history, the blood picture and the results of the pathologic-anatomic examinations justify the diagnosis of agranulocytosis. There are no indications that this agranulocytosis should have had an exogenous toxic or allergic origin.

Case T9

Girl. Born July 21, 1953. Died September 3, 1953. Normal delivery. Weight at birth, 3200 g. Vaccinated against tuberculosis. She was breast-fed. At the age of five days, a pea-sized, reddish-blue, elevated efflorescence appeared above the right ear. After a few days, pus mixed with blood was discharged. Penicillin ointment was prescribed. The sore continued to discharge. At the age of two weeks, she was taken to the children's ward of Gällivare Hospital and was treated there for ten days. Upon arrival, she was thin but otherwise in a good general state. She had a pronounced stomatomycosis with white deposits on the buccal mucosa and tongue. Within an area of 2 × 1 cm just above the navel, the skin was swollen and miscolored and bluish-red. In the scalp immediately above the right ear, she had an oval, bluish-red swelling about 1.5 × 2 cm in size. This bordered on a bluish miscolored skin area with a diameter of about 2½ cm, where the skin appeared thin and partly eroded. A light red streak stretched down the swollen cheek. Close to the median line in the left fronto-parietal region, a pea-sized, cyanotic, furuncle-like formation could be seen. Her tempera-

ture was normal. The internal organs were normal. White blood cells 8100 per cu.mm. Differential count: segmented granulocytes 17 per cent; stab cells 8 per cent; eosinophils 0 per cent; basophils 0 per cent; lymphocytes 55 per cent; monocytes 20 per cent. She was treated with penicillin and the local skin changes regressed. The swelling above the right ear decreased. A skin infiltration in the cheek could be felt corresponding to the parotid gland. This infiltration still remained when she was discharged from the hospital. At a control examination three weeks later, the skin above the ear and on the cheek was normal. Small areas with folliculitis were scattered around the neck and in the right groin.

At a new control September 1, 1953, she had a large abscess on the right side of the neck and a smaller one in front of the right ear. She had scattered areas of folliculitis all over the body. She looked greyish-pale and sickly and had a temperature of 39.5°C. On the right side of the neck and in the shoulder region, she had large abscesses which had partly emptied themselves. Around the neck and in the armpit there were a great number of small areas of folliculitis. Such areas were also found in skin folds on other parts of the body. She had a red spot and swelling in front of the right ear. Her weight was 2960 g. Blood: Hemoglobin 97 per cent; erythrocytes 5 million; white cells 10,800 per cu.mm. Differential count: segmented granulocytes 4 per cent; stab cells 0 per cent; eosinophils 0 per cent; basophils 0 per cent; lymphocytes 80 per cent; monocytes 16 per cent. This preparation was later checked by N. G. Nordenson, M.D., with the following result: Segmented granulocytes 0.5 per cent; lymphocytes 26.5 per cent; monocytes 73 per cent. Dr. Nordenson's diagnosis was agranulocytosis with mainly monocytous reaction. The monocytes did not appear directly pathological.

She was treated with penicillin. A few days later, a sudden change for the worse took place, and she died.

Pathologic-Anatomic Examination **(Fredrik Wahlgren, M.D.)**

The acinous architecture of the *liver* (Fig. 8a) could not be distinctly seen. The liver cells were enlarged. Large spaces were seen in the cytoplasm, particularly in the cells of the peripheral acini, probably resulting from dissolution of fat. The capillaries between the sini were compressed and empty. In the central parts of the acini, however, a small number of red or white corpuscles could be seen. The majority of the Kupffer astral cells were enlarged. Some of them contained a fine-grained, brown pigment, probably an iron pigment. The gall bladder and ducts were normal. The follicles of the *spleen* (Fig. 8 b, p. 26) were small and few in number. They lacked germinal centers. The pulp was rather poor in blood, but rich in cells. The reticulum was distinctly increased. In the pulp, numerous large phagocytes carrying iron pigment were found. The vessels and the trabecular system appeared essentially normal. The *thymus* was remarkably poor in cells and contained very few lymphocytes. The reticular cells were enlarged. Only few Hassal's corpuscles were found. A *lymph node*, the size of a

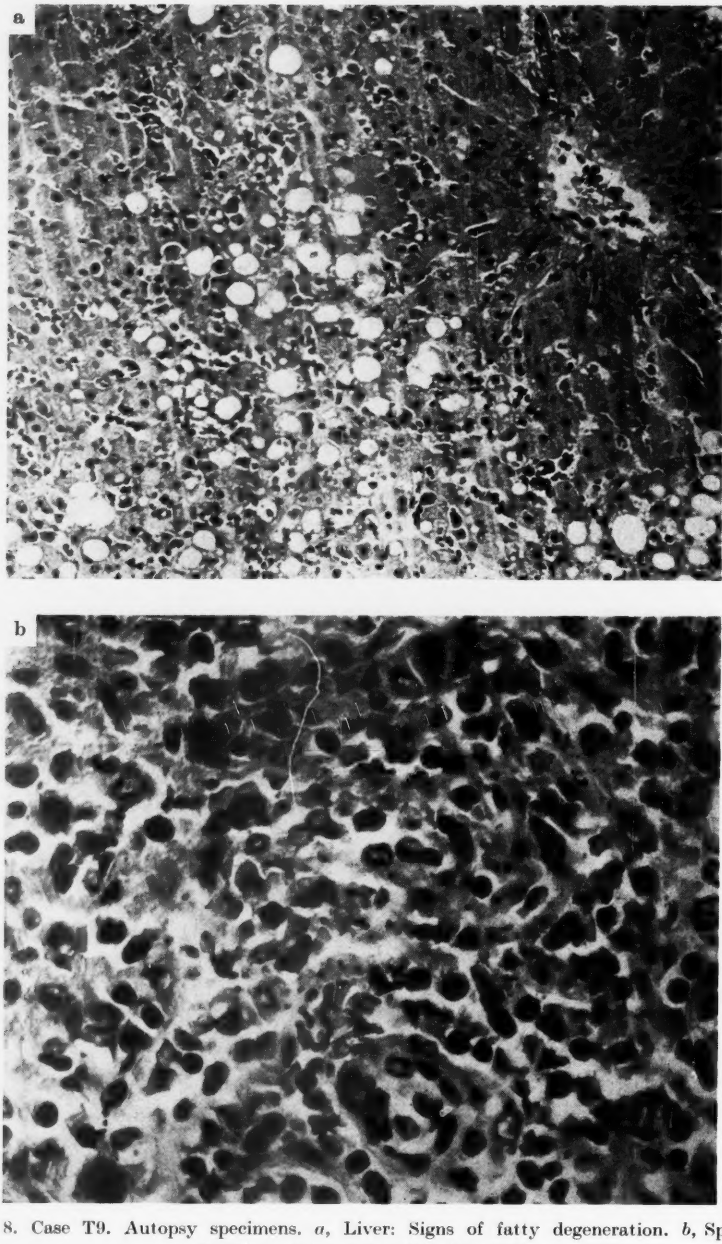


Fig. 8. Case T9. Autopsy specimens. *a*, Liver: Signs of fatty degeneration. *b*, Spleen: Swelling of the sino-endothelial cells.

hemp seed, showed a distinct enlargement of the sinus endothelium and an increase of the reticulum. The lymphocytes were considerably fewer than normal. Scattered eosinophils and phagocytes carrying an iron pigment could be seen. The *adrenal glands* were normal. The *bone marrow* was rather poor in cells, but, as far as could be judged without making a detailed counting of the cells, the distribution of the different cell types appeared normal. No obvious enlargement of the reticular cells could be observed.

The major findings in this case were an enlargement of the endothelial cells and an increase of the reticulum in the spleen and the lymph nodes. This indicates an increased breakdown of erythrocytes, but does not explain its cause. The reduction in the number of lymphocytes in different organs was remarkable.

Epicritic Summary

A girl fell ill at the age of three weeks with a phlegmon in the scalp. She had no fever. Blood examinations showed granulocytopenia and a strong shift to the left within the peripheral white blood cells. She was treated with penicillin and improved. After another month, she became gravely ill, had high fever and a few large abscesses on the neck. Blood examination showed agranulocytosis. She was again treated with penicillin, but died after a few days, at the age of two months.

The clinical course, the blood examinations and the result of the pathologic-anatomic examination justify the diagnosis of agranulocytosis. There were no indications that this agranulocytosis should have had an exogenous toxic or allergic origin.

Case R4

Boy. Born June 15, 1951. Weight at birth, 3200 g. He was breast-fed for 4 months, after which a supplement of gruel was given and, after the age of 7 months, he got a mixed diet. When he was $2\frac{1}{2}$ months old, an abscess in the one gluteal region was treated by a physician with an incision and penicillin. The wound healed in two weeks. After an additional two weeks, another abscess on the right side of the trunk was treated with penicillin. At the age of four months, he got another abscess the size of a walnut on the scalp and several small furuncles on the neck. At this time, he had also high fever. He was taken to the hospital in Kalix, received aureomycin, and was discharged as recovered. At the age of seven months, a furuncle appeared in the right auditory canal. He was again taken to the Kalix Hospital and received aureomycin for two weeks. Upon returning home, he was pale, fussy, perspired much, and had occasional fever. One month later, new abscesses in the scalp appeared. He was again treated with aureomycin at home. His temperature became normal and the abscesses healed.

At the age of ten months, he had fever again and new abscesses on the scalp. He was taken to the pediatric clinic in Boden. Upon admission, he was febrile and had a dirty-pale skin color. He was thin and his tonus and turgor were decreased. There were several furuncles and abscesses of varying size on the scalp and neck, the largest one being 5×5 cm (Fig. 9). In the center of this abscess there was a bluish-red necrotic spot, covered by a crust. On the neck, there was an abscess the size of a walnut in the process of drying. There were radiate scars from earlier abscesses in several places on the scalp, neck, gluteal regions and the trunk. On the neck were several bean-sized, firm adenites and on both groins, pea-sized adenites. The liver was palpable, but not noticeable enlarged. The spleen could not be felt. Other internal organs appeared normal upon physical examination.

The white blood cells showed an almost complete agranulocytosis. A bacteriological blood culture was negative. Cultures from abscesses gave growth of a pathogenic strain of Staphylococcus aureus. Antistreptolysin titer 22 units per ml. Antistaphylosin titer 28 units per ml. He was treated with penicillin and chloromycetin, and became free of fever in ten days. On April 27, 1952, he was referred to the pediatric clinic of the University Hospital, Uppsala, for continued treatment and special examinations. He was under treatment and observation there until October 28, 1952.

During the whole time, he had complete agranulocytosis (Fig. 10, p. 30). The bone marrow showed a block in maturation at the myelocyte stage. X-ray examinations of the skeleton, heart and lungs showed normal conditions. The total blood protein quantity was normal, but there was a great increase in γ-globulin (42 per cent). Wassermann reaction in blood was negative. Bacteriological cultures from abscesses gave growth of Staphylococcus aureus.

Attempts to improve the bone marrow with di-menformone, folacin, pernaemon forte with vitamine B_{12}, and cortisone were of no effect.

Cell culture experiments with bone marrow were performed (cf. p. 34). Activation and maturation of the cells were obtained when cysteine or normal serum was added. As a therapeutic trial, cysteine was given oraly, subcutaneously and as intravenous drip, but without effect.

During the entire stay in the hospital, he obtained, in different periods, large doses of chloromycetin, penicillin, elkosin, and streptomycin. On several occasions, he had remittent fever, as a rule without other objective findings. On one occasion, he had a severe balanitis, and, on another occasion, an abscess following an injection of pernaemon.

The general development of the boy was retarded. He increased but slowly in weight (7140–8750 g.). During the entire stay in the hospital, he had temper tantrums almost daily, and often several times a day. The

Fig. 9. Case R4. *a*. Abscesses of the neck. *b*, Microphotograph of bone marrow smear.

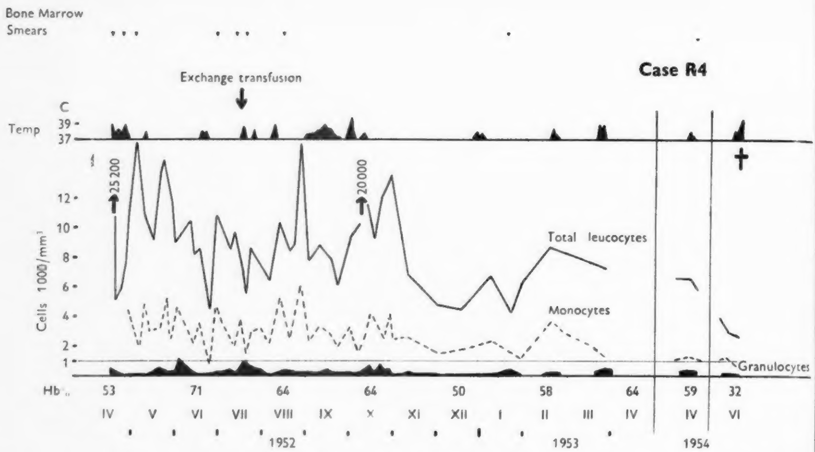


Fig. 10. Case R4. Graphical representation of the blood values during the course of illness.

psychic development otherwise appeared relatively normal. His electroencephalogram was normal.

After discharge from the hospital in Uppsala (October 28, 1952), he was treated in his home. During 1953 and 1954, he had numerous infections of different kinds, anginas, septic-necrotic sores in the mouth, cutaneous abscesses and bronchial pneumonias. He was treated with antibiotics by the district physician and, on several occasions, at the Children's Hospital in Boden. The blood examinations constantly showed agranulocytosis. His general condition gradually became worse. Cramp attacks of a more genuine grand mal character were observed occasionally.

In July 1954, he was again hospitalized with extensive bronchial pneumonias, and died after a few days on July 28, 1954. He was then three years and one month old.

Special Examinations

Blood examinations have been summarized in Table II and Fig. 10.

Bone Marrow Examinations

April 16, 1952: (N. G. Nordenson, M.D.) Sternal punctate: Cell-rich preparation. Myelopoiesis was totally blocked after promyelocytes. In addition, a maturation disturbance in erythropoiesis was observed. The red blood cells showed a marked anisocytosis. The megakaryocytes were normal. The reticulum was strongly hyperplastic with an increase in lymphoid elements.

Diagnosis: Probably an agranular reaction. Suspected leukemia.

Table II

Case R4. Blood examinations.

Date	Hemoglobin %	Red blood cells million per mm³	Reticulocytes per 1 000	White blood cells per mm³	Stab cells %	Segmented neutrophils %	Eosinophils %	Basophils %	Lymphocytes %	Monocytes %	Plasma cells %	Platelets per mm³	Sedimentation rate
1952													
16/4	53	3.46		25,200	0.5	1.5	—	—	56	42	—	227,000	61
19/4	49	3.26		5,100	—	—	—	—	93	7	—		
23/4		3.86		6,000									65
25/4	55	3.84		7,700	—	—	—	0.5	95.5		—		
28/4	60	3.75	30	11,800	—	—	0.5	—	58	38	3.5	246,000	44
2/5	63	3.40		16,200									57
3/5					—	0.5	—	1	50	48	0.5		
6/5	57	3.75		6,700	—	0.5	—	1.5	66.5	29	2.5		59
9/5	53	3.10		10,900	—	2	—	1	48	49	—		53
13/5	52	3.60		10,200	—	—	1	1	68	30	—		52
15/5	53	3.47	12	9,200	—	—	2	1	61	36	—		58
17/5			62										
20/5	52	3.56	56	13,600	—	—	2.5	1.5	72.5	23.5	—		38
22/5	56	3.49	32	14,600									40
24/5			20	13,100	—	—	5	1.5	54	39.5	—		
27/5	58	3.56	18	11,800	0.5	0.5	1	0.5	77.5	20	—		30
29/5			10										
30/5	59	3.48		9,000	—	0.5	2	0.5	75	22	—		26
1/6			6										
3/6	62	3.61	11	19,000	—	—	5	1	69.5	24.5	—		31
10/6	58	3.02	4	10,400									30
13/6	58	3.75	10	8,200	—	0.5	2	—	72	25.5	—		39
17/6	71	4.06	10	8,300	0.5	1	—	0.5	55.5	42.5	—		
24/6	56	3.52	22	4,500	—	—	1	0.5	80.5	17	1		32
28/6	56	3.82	50	10,600	—	0.5	0.5	1.5	53	44.5	—		31
1/7	61	4.02	24	10,600	—	1	2	—	68.5	28.5	—		42
3/7	59	3.92	52	9,600	—	2	1.5	—	59.5	37	—		
8/7	54	3.88	32	8,600	—	3	1.5	1	62	32	—		24
11/7	57	3.76	12	9,500	—	—	2.5	1	74.5	21	1		35
13/7			14										
15/7	61	4.24	24	23,300	0.5	—	2	—	81	16.5	—		16
16/7	exchange transfusion												
17/7	73	3.30	42	7,000	1.5	3	9	—	78.5	8	—	302,000	7
1[illegible]/7	70	3.32		5,600	—	—	16.5	1.5	52.5	26	3		
2[illegible]/7	67	3.57	24	8,600	—	0.5	7.5	—	56.5	34.5	1		38
2[illegible]/7	65	3.28	26	7,300	—	1.5	2	2	45	44.5	5		40

(*cont.*)

(Table II, cont.)

Date	Hemoglobin %	Red blood cells million per mm³	Reticulocytes per 1000	White blood cells per mm³	Stab cells %	Segmented neutrophils	Eosinophils %	Basophils %	Lymphocytes %	Monocytes %	Plasma cells %	Platelets per mm³	Sedimentation rate
5/8	65	3.20		6,300	0.5	—	1	1	61	34.5	2		36
12/8	64	3.78	10	11,400	—	0.5	2	0.5	51	46	—		43
19/8	64	3.86	22	8,300	—	1	1.5	0.5	66.5	30.5	—		27
22/8	65	3.56	24	8,900	—	—	1.5	0.5	60	37.5	0.5		
26/8	64	3.25	26	15,800	—	—	—	1.5	60	38.5	—		36
2/9	62	3.20	26	7,900	—	1	2	1	66.5	28.5	1		43
9/9	52	2.53	18	8,800	0.5	1.5	—	1	59.5	37.5	—		53
16/9	53	2.80	14	8,000	—	1.5	1.5	2.5	56	37	1.5		34
22/9	54	3.08	28	6,200	—	1	—	3.5	58	30.5	1		27
30/9	57	3.26	20	9,300	—	—	—	1.5	62	36.5	—		
7/10	53	3.30	16	10,300	—	—	0.5	1	80.5	16	2		
10/10	59	3.15	30	15,500	—	—	—	—	84.5	15.5	—		34
14/10	54	3.41	30	18,600	—	—	3	1	74.5	21.5	—		26
17/10	64	3.50	20	9,300	—	1.5	1	0.5	65	32	—		34
21/10	62	3.28	8	20,000	—	—	1.5	2.5	81	15	—		28
24/10	63	3.62	18	11,900	—	0.5	0.5	1	78.5	19.5	—		26
28/10	65	3.30	10	13,600	—	—	3	0.5	63.5	32	1		42
30/10	49	3.00		5,100	—	0.5	2.5	0.5	46.5	50	—		
6/11	57												
10/11	54			6,900	—	3	1	—	57	38	—		
12/11	56												
19/11	55												
26/11	50												
1/12	47	3.06		4,900	1	0.5	—	—	66	32.5	—		
10/12	54												
17/12	50			4,500									
1953													
6/1	56												
7/1	54	3.52		6,700	1	1	—	—	63	35	—		
8/1					—	4	0.5	—	71.5	21	3	(Nordenson)	
14/1	58												
21/1	54	3.60		4,300	—	6	4	—	57	33	—		
28/1	63	3.84		6,200	—	0.5	1	0.5	78	20	—		
18/2	53	3.63		8,700									6[illegible]
19/2			4	8,600	—	1	—	2	53	43	—	34,500	
26/3		3.12		7,300	—	4	1	2	75	18	—		7[illegible]
8/4	64												

(cont.)

(Table II, cont.)

Date	Hemoglobin %	Red blood cells million per mm³	Reticulocytes per 1000	White blood cells per mm³	Stab cells %	Segmented neutrophils %	Eosinophils %	Basophils %	Lymphocytes %	Monocytes %	Plasma cells %	Platelets per mm³	Sedimentation rate
1954													
3/4	59	3.64		6,600	—	2	3	2	72	20	—		53
5/4			1									284,000	
13/6	39	2.49	1	5,000	1	1	—	—	75	22	—	196,000	63
14/6	38	2.48	6	4,300									
18/6	32	1.75		4,800	—	1	—	—	85	14	—		
22/6	29												

April 23, 1952. (N. G. Nordenson, M.D.) Relatively cell-rich preparation. The normal structure was completely erased, and the picture was dominated by monocytes, monocytoids, lymphocytes (partly pathologic), plasma cells and reticulum cells.

Diagnosis: Agranular pattern, now less leukemic than at the previous examination.

May 2, 1952. Myelogram (Professor B. G. Vahlquist):

	Per cent
Myeloblasts	0.2
Promyelocytes	—
Myelocytes: neutrophilic[1]	55.8
eosinophilic	2.0
basophilic	0.2
Metamyelocytes: neutrophilic	1.2
eosinophilic	0.8
basophilic	—
Stab cells: neutrophilic	0.4
eosinophilic	—
basophilic	—
Polymorphonuclear neutrophils	0.2
eosinophils	0.4
basophils	—
Normoblasts: acidophilic	2.4
basophilic	9.4
Megakaryocytes	0.2
Reticuloendothelial cells	3.4
Ferrata cells	—
Monocytes	1.0
Lymphocytes[2]	19.8
Plasma cells	5.4

[1] Partially large, vacuolated.

[2] Many atypical, resembling fibroblasts.

June 28, 1952. Tibia punctate (F. Nordbring, M.D.): Myelopoiesis was blocked approximately at the myelocyte stage. Singel cells matured as far as to stab nucleated cells could, however, be seen. The reticulum was hyperplastic. Erythropoiesis appeared retarded.

July 11, 1952. Sternal punctate (F. Nordbring, M.D.): The pattern was essentially the same. A few metamyelocytes, single stab nucleated cells and an occasional mature leucocyte could be seen. The lymphocytes were increased in number. Erythropoiesis appeared somewhat more active.

July 19, 1952. Sternal punctate (F. Nordbring, M.D): On comparison with earlier preparations prior to an exchange blood transfusion on July 16, 1954, this preparation showed more stab nucleated and segmented nucleated cells; the latter were predominantely eosinophilic. Eosinophilic myelocytes and metamyelocytes were also numerous. Erythropoiesis appeared more active than before. Otherwise, lymphocytous and reticulous elements dominated as in earlier preparations. Several plasma cells were seen.

January 8, 1953. Sternal punctate (N. G. Nordenson, M.D.): The pattern was, in principle, the same as on previous occasions. A certain improvement appeared to have taken place, since the myelopoiesis showed more mature forms.

Diagnosis: Granylocytopenia. Reticulopathy.

April 10, 1954. Tibial punctate (N. G. Nordenson, M.D.): Cell-poor preparation. The pattern showed a dominance of lymphatic, monocytous, and reticulous elements.

Special Examination of the Bone Marrow including Cell Culture Experiments (C. Munk Plum, Ph.D., Copenhagen)[1]

Bone marrow smear preparations: The bone marrow displayed a complete inhibition of myelopoiesis at the myelocyte stage. A number of more or less atypical mitoses could also be observed. Thus, some tetraploid mitoses and, probably as a consequence of this, atypical forms of nuclei were also found. The monocytes and especially the plasma cells appeared to be increased. Certain parts of the preparation resembled the findings in myelomatosis.

(1) Culture of the patient's bone marrow cells in his own serum: After the course of 24 hours with determinations at 0, 3, 6, 9 and 24 hours, it was found that, despite a slight increase in the number of immature precursors, the regeneration of erythrocytes calculated per normoblast was somewhat lower than normal, 4.23 compared to the normal of 5.39, i.e. 79 per cent of normal activity. No maturation of the myelopoietic cells could be observed.

(2) Culture of the patient's bone marrow cells in the serum of a normal individual: An increased activity of the normoblasts was found. The regeneration per normoblast was 4.79 with one normal serum and 4.84 with another. This regeneration is remarkable since earlier investigations had shown that, in culturing normal marrow in the individual's own serum and normal marrow in normal serum from another individual, the rate of erythropoiesis is about 15 per cent lower in the latter case.

Already after 9 hours, the myelopoietic cells showed signs of developing in to more mature forms, as now a few metamyelocytes and single stab-nucleated, neutrophilic granulocytes could be observed. This maturation was still more pronounced after 24 hours.

[1] Personal communication by Professor Bo C. Vahlquist.

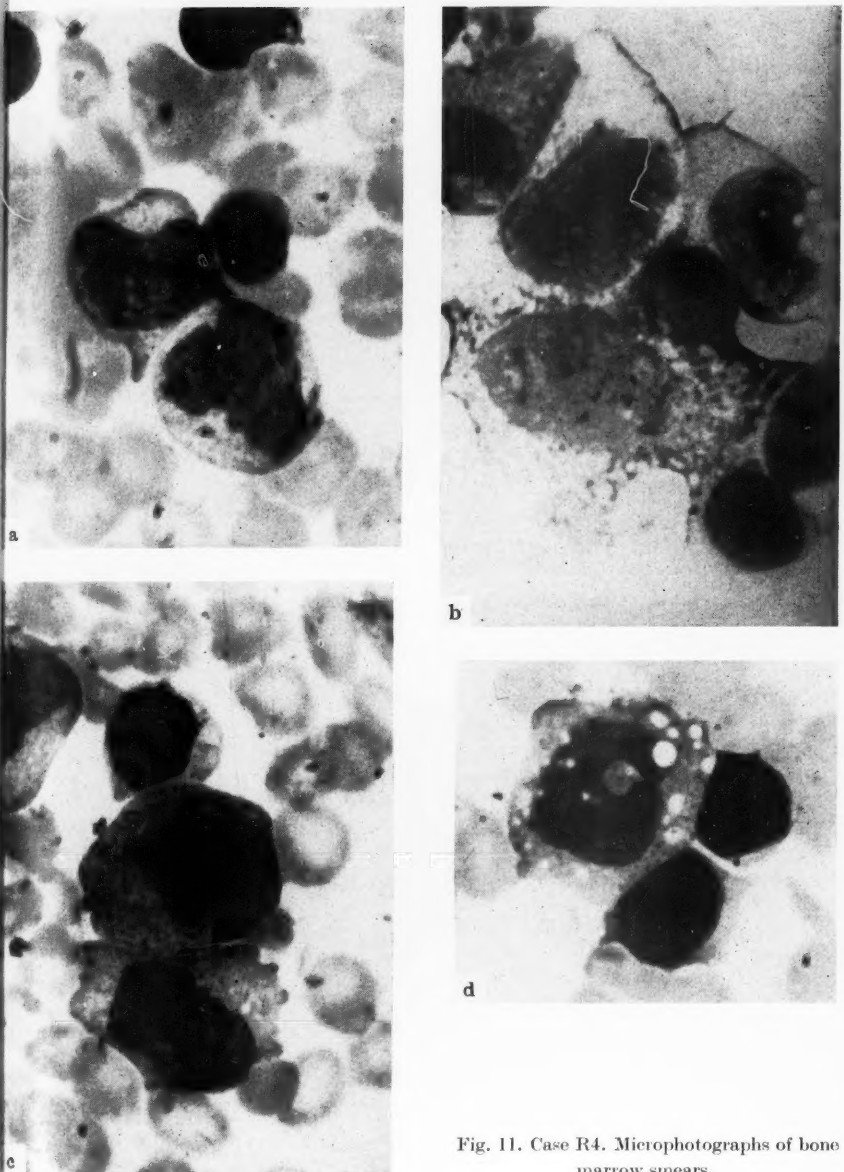


Fig. 11. Case R4. Microphotographs of bone marrow smears.

(3) Culture of normal bone marrow cells in the patient's serum: A slight decrease in the rate of erythropoiesis should be expected. However, this decrease was considerably greater than anticipated. Consequently, the patient's serum had either an inhibitory effect or lacked substances required to maintain a normal rate of erythropoiesis.

(4) Culture of the patient's bone marrow cells in his own serum with added tyrosine: This experiment gave the same result as experiment (1).

(5) Culture of the patient's bone marrow cells in his own serum with added cysteine: The cysteine was added in amounts equivalent to 0.3 mg per 100 ml. blood. In this experiment, completely normal erythropoiesis was observed, and the differentiation in the cells of myelopoiesis was more pronounced than when using the patient's own serum alone. The normalization was more pronounced than when normal serum was used.

(6) Culture of the patient's bone marrow cells in normal serum with added cystein: The result was about the same as under (5).

Conclusion: In culturing the patient's bone marrow cells, a normalization of the cell development and maturation was observed when cysteine was added to the patient's serum, and a relative normalization when cultivation occurred in serum from a normal individual.

These in vitro experiments suggest that the patient could not fully utilize the sulfur-containing amino acids that are of importance in cell differentiation.

X-ray examination of heart and lungs, April 17, 1952. Normal findings.

X-ray examination of skeleton, April 30, 1952. Posteriorly and parietally on the right side of the skull, there was a compact condensation the size of a pea. All other skeletal structures appeared normal.

Serological tests, May 7, 1952. *Wassermann* reaction in blood negative. *Antistreptolysin titer* 22 units per ml. *Antistaphylosin titer* 28 units per ml. May 12, 1952. Antistaphylosin titer 5.6 units per ml.

Blood proteins, April 30, 1952. Total proteins as measured by Kjelldahl 8.5 per cent. The γ-globulin fraction was 42 per cent of total protein (fig. 12). May 17, 1952: Total proteins 8.6 per cent, thereof albumin 3.2 per cent and globulin 5.4 per cent. June 14, 1952: Total proteins 7.5 per cent. August 14, 1952: total proteins 7.5 per cent (γ-globulin 29 per cent (fig. 12)). On March 13, 1953: Total proteins 7.5 per cent.

Biopsy of bone marrow, May 9, 1952 (N. Gellerstedt, M.D.): The bone marrow was rich in cells, with moderate erythropoesis and rather active myelopoesis; however, the more mature stages were poorly represented. Approximately normal numbers of megakaryocytes were observed. There was no distinct proliferation of the reticular cells.

A *lymph node* excised at the same time showed chronic inflammatory reticulosis of a common type.

The findings gave no conclusive information regarding the nature of the disease.

Pathologic-Anatomic Examination **(Fredrik Wahlgren, M.D.).**

Within the examined parts of the *lungs*, there were wide-spread pneumonias which showed a pronounced tendency to necrosis and formation of abscesses. The necrotic masses were extremely rich in various kinds of bacteria, mostly Gram-positive cocci

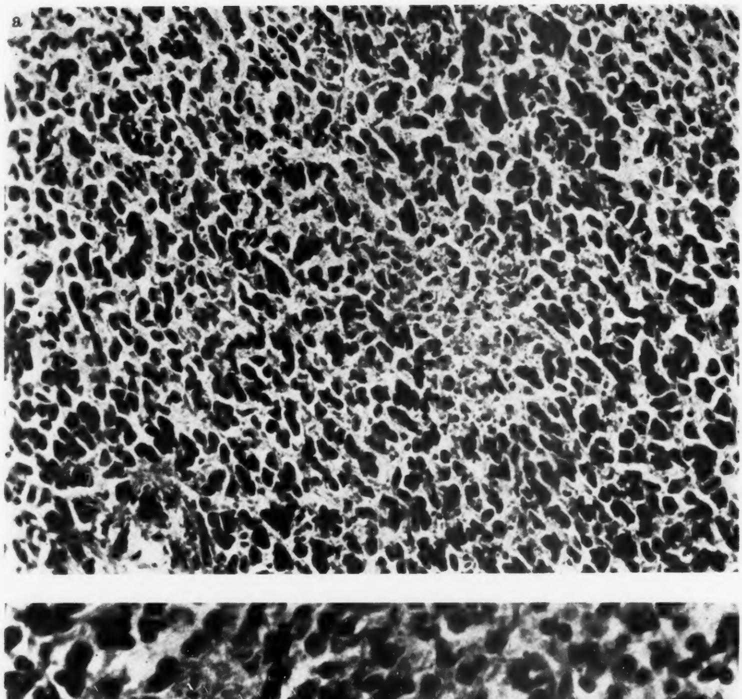


Fig. 13. Case R4. Autopsy specimens: *a*, Liver: Focus with necrotic and necrobiotic cells. Depletion of acinar structure. *b*, Spleen: Swelling of the sino-endothelial cells.

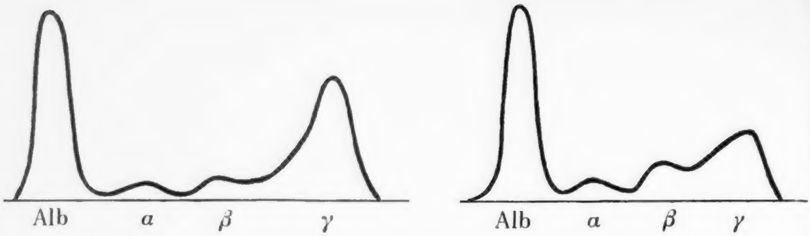


Fig. 12. Case R4. Blood proteins by electrophoresis.

30/4 1952.

Electrophoresis

Albumins	42 %
Alpha-globulin	5 %
Beta-globulin	10 %
Gamma-globulin	42 %

$\frac{\text{Albumins}}{\text{Globulins}} = 0.72$

14/8 1952.

Total proteins . . . . 7.5 %

Electrophoresis

Albumins	49 %
Alpha-globulin	7 %
Beta-globulin	15 %
Gamma-globulin	29 %

$\frac{\text{Albumins}}{\text{Globulins}} = 0.96$

in large clumps. In addition, there were cocci and bacilli having a different formation. No acid-fast bacteria were seen.

The acinous architecture of the *liver* (Fig. 13a) appeared distinct. The liver cells in the central parts of the acini were largely necrotic or more or less strongly necrobiologically changed. In the periphery of the acini, the liver cells were wide and empty. The periportal connective tissue was more abundant than usual. There was no pathological concentration of cells in it. Gall passages and vessels appeared normal.

The follicles of the *spleen* (Fig. 13b) were small. They lacked germinal centers and their borders towards the pulp were rather diffuse. The pulp was devoid of blood and rich in cells. The pulp cells were not enlarged and there were no pathological cell elements in the pulp. The reticulum appeared to be somewhat increased.

The normal architecture of the *lymph nodes* was diffuse without being completely erased. The tissue contained a few enlarged reticular cells. Giant cells or eosinophilic leucocytes were not observed. The *bone marrow* was relatively poor in cells. It contained predominantly erythrocytes and their precursors. The reticulum cells appeared enlarged. There was only a small number of megakaryocytes.

Epicritic Summary

A boy, who, from the age of 2½ months, repeatedly had furuncles and abscesses of the skin in connection with high fever. He was treated with antibiotics. No examination of the white blood cells was undertaken until he was 10 months old. He then had numerous furuncles and abscesses again, as well as high fever. The blood showed agranulocytosis and monocytosis. Bone marrow examinations showed a maturation block at the promyelocyte-

ERRATUM

It is regretted that Fig. 12, p. 38, has been placed *after*, and not *before*, Fig. 13, p. 37.

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myelocyte stage. The continued course of the disease was characterized by recurring septic skin conditions and infections of the upper respiratory tract. The infections could be counteracted with antibiotics, but recurred as soon as the treatment had been discontinued for some time. Despite the infections and the pronounced granulocytopenia-agranulocytosis which persisted throughout the course of the disease, he could, with the aid of antibiotic treatment, be kept alive until the age of three years, when he died of widespread broncho-pneumonias.

The clinical course, the blood and bone marrow pictures, as well as the post mortem findings, justify the diagnosis of agranulocytosis. There were no indications that this agranulocytosis should have had an exogenous toxic or allergic origin.

Case K2

Boy. Born June 15, 1950. Weight at birth, 3600 g. Normal delivery. Breast-fed for 2½ months. On September 9, 1950, he was taken to an infirmary because he was pale and looked sick. He had a moderate stridor. The temperature was subfebrile, but there were no obvious signs of infection. He had a slight anemia and was treated with iron. A sternal puncture was made on September 18, 1950. The preparation showed maturation arrest in both myelopoesis and erythropoesis. Therefore, the child was transferred to the Children's Hospital in Boden on September 20, 1950. He was pale in spite of a hemoglobin value of 87 per cent. He had a slight stridor of a predominantly inspiratory type. There were no signs of rickets. The liver, spleen and superficial lymph nodes could not be felt. The internal organs appeared normal on physical examination.

Since the bone marrow continued to show the same type of changes as at the first examination, he was observed and treated in the hospital until November 20, 1950, when he was discharged at the request of his parents. During the entire stay at the hospital, he had a dirty-grey skin color. The paleness was in no way correlated with his hemoglobin values. On some occasions, the blood showed relative granulocytopenia. The lowest absolute number of granulocytes was 900 per cu.mm. Concerning the continued course, it is only known that the child was still alive in 1954.

Special Examinations

Blood examinations have been summarized in Table III.

Bone Marrow Examinations (N. G. Nordenson, M.D.)

September 18, 1950. Sternal punctate: Very cell-rich preparation. A slight maturation arrest in myelopoiesis with an increase in myelocytes and promyelocytes. Slight eosinophilia. Erythropoiesis was hyperplastic with strong maturation arrest; increase

Table III

Case K2. Blood examinations.

Date	Hemoglobin %	Red blood cells million per mm^3	White blood cells per mm^3	Stab cells %	Segmented neutrophils %	Eosinophils %	Basophils %	Lymphocytes %	Monocytes %	Unspecified cells %	Platelets per mm^3	Sedimentation rate
18/9				1	10.5	2.5	—	83.5	2.5	—		
21/9	80	3.84	5,100	1	16	4	—	70	9	—	278,000	15
23/9											278,000	
25/9				0.5	20.5	—	—	72	7	—		
27/9	88		6,700	1.5	26	3	—	64	5	0.5		12
29/9			7,700	1	22	3	—	70	4	—		
2/10			8,300	0.5	28	—	—	61	7.5	1		11
4/10	95		9,900	—	25.5	2	0.5	67.5	4.5	—		
16/10	91		11,200	—	28	4	—	62	6			
27/10	84		11,000	1.5	28.5	1.5	—	65.5	3	—		
3/11	92		6,500	—	12	5	—	73	10	—		
8/11	94		10,000	1	18	—	—	76	5	—		
15/11	88	4.26	6,700	1	21	5	—	65	7	1		
17/11			7,400	—	10	2	0.5	83.5	4	—		
20/11			8,700	—	29	2	1	66	2	—		

21/9 Protrombin index 93 Bleeding time: 2 min 40 sec
Clotting time: 3 min 40 sec

in basophilic normoblasts and proerythroblasts. Strong maturation disturbance with anisocytosis. The megakaryocytes showed degenerative features. There was a strong increase in lymphocytes without definite metaplasia. The reticulum was very hyperplastic.

Diagnosis: Strongly reactive bone marrow with signs of chronic irritation. No specific features. Probably a systemic disease.

September 25, 1950. Sternal punctate: Very cell-rich preparation. Strong maturation arrest in myelopoiesis; increase in myelocytes and promyelocytes. Erythropoiesis was hyperplastic with maturation arrest; increase in basophilic normoblasts and proerythroblasts. Slight maturation disturbance. Megakaryocytes were scanty. Strong increase of lymphocytes of an immature and pathologic type. The reticulum was very hyperplastic.

Diagnosis: Very strongly reactive marrow. Chronic irritation. Leukemia-like picture. Systemic disease?

Ocotber 4, 1950. Sternal punctate: Cell-rich preparation. A slight maturation arrest in myelopoiesis with an increase in myelocytes. Erythropoiesis was slightly hyperplastic with some maturation arrest and maturation disturbance. The megakaryocytes

showed slight maturation arrest. Increase in lymphocytes without a definite metaplasia. Reticulum very hyperplastic.

Diagnosis: Strongly reactive marrow. Chronic irritation. Systemic disease?

Tibial punctate: Very cell-rich preparation. Slight maturation arrest in myelopoiesis with an increase in myelocytes. A certain maturation block after stab nucleated cells. Erythropoiesis was slightly hyperplastic with some maturation arrest and maturation disturbance. Megakaryocytes with slight maturation arrest. Lymphocytes strongly increased; possibly metaplasia. Reticulum very hyperplastic.

Diagnosis: Strongly reactive marrow. Chronic irritation. Systemic disease? Lymphatic leukemia?

October 21, 1950. Tibial punctate: Very cell-rich preparation. Strong maturation arrest in myelopoiesis down to myeloblast-stem cells. Moderate maturation block after stab nucleated cells. Erythropoiesis was hyperplastic with maturation arrest; increase in basophilic normoblasts and proerythroblasts. Slight maturation disturbance. Megakaryocytes were normal. Strong increase in lymphocytes, but no metaplasia. Reticulum strongly hyperplastic.

Diagnosis: Reactive marrow. Systemic disease?

November 1, 1950. Tibial punctate: Cell-rich preparation. Strong maturation arrest in myelopoiesis with increase in myelocytes and promyelocytes. Maturation block after stab nucleated cells. Erythropoiesis was hyperplastic with strong maturation disturbance. Megakaryocytes were normal. Lymphocytes were strongly increased in number but showed no metaplasia. The reticulum was strongly hyperplastic.

Diagnosis: Reactive marrow. Some granulopenia. Systemic disease?

November 13, 1950. Tibial punctate: Very cell-rich preparation. Strong maturation arrest in myelopoiesis with increase in myelocytes and promyelocytes, as well as in myeloblast-stem cells. Erythropoiesis was hyperplastic with moderate maturation arrest. Megakaryocytes were normal. The reticulum was strongly hyperplastic.

Diagnosis: Reactive marrow. No specific features. Systemic disease?

X-ray examination of the skeleton, September 22 and November, 1950: No pathological changes.

Wassermann reaction in blood: negative. *Mantoux* 1 mg.: negative.

Epicritic Summary

A boy, because of paleness and relatively poor general state of health, was taken to a hospital at the age of three months. He then had a moderate congenital stridor. He was observed for a period of two months. On some occasions, he had a moderate granulocytopenia. The lowest absolute number of granylocytes was 900 per cu-mm. During the entire period, the bone marrow examinations disclosed maturation disturbances or maturation arrest in myelopoiesis, as well as in erythropoiesis. It was remarkable that, despite this disturbance in erythropoiesis, the blood gave normal values for hemoglobin and the number of erythrocytes.

Concerning the continued course, it is only known that the child was still alive in 1954.

The blood and bone marrow findings make it probable that there was a disturbance in myelopoesis of a granulocytopenic-agranulocytotic type, in principle, similar to the one observed in the other cases described within Group I. It seems likely that this boy suffered from the same disease, but that it occurred in a less severe form.

Case L1

Boy. Born January 31, 1950. Weight at birth 2760 g. He was breast-fed for two months, and developed normally. At the age of five months, he fell ill with fever and got several furuncles on the scalp and neck. The largest ones were of the size of hazel nuts. He was taken to an infirmary and treated there with incisions and penicillin. He became free of fever and the skin processes healed. His hemoglobin was 79 per cent. A few days after discharge, he again developed two furuncles the size of hazel nuts on the neck. These healed without treatment. Because of insufficient increase in weight and poor general state of health, he was, however, again taken to the infirmary. On October 25, 1950, a differential count of the white blood cells showed a considerable granulocytopenia with only 4.5 per cent granulocytes. The patient was transferred to the Children's Hospital in Boden on November 16, 1950. On November 26, 1950, he was sent to the Sachs' Hospital for Children in Stockholm for continued treatment and observation.

At the time of the first examination in the hospital, he had fever and signs of an infection in the respiratory tract. He soon became afebrile, and from then on the temperature was normal. During the two months of observation, his general state of health was relatively good. However, he increased poorly in weight. The weight was about 8500 g. There were no signs of rickets. He was anemic and was treated with iron. His hemoglobin rose from 46 to 75 per cent. The liver and spleen were not palpable, and there was no enlargement of the lymph nodes. With the exception of the initial granulocytopenia, the white blood cells did not exhibit any noteworthy changes. During the whole period, sternal punctates showed a reactive marrow with a maturation arrest in myelopoiesis with an increase in myelocytes and promyelocytes. Erythropoiesis showed signs of maturation disturbance, and the reticulum was strongly hyperplastic.

After discharge from the Sachs' Hospital for Children on January 3, 1951, he returned home. Concerning the continued course, it is only known that he was still alive in 1954.

Special Examinations

Blood examinations have been summarized in Table IV.

Bone Marrow Investigations (N. G. Nordenson, M.D.)

October 25, 1950. Sternal punctate: Very cell-rich preparation. There were signs of toxic myelopoiesis and strong maturation arrest down to myeloblasts. The eosinophilic cells were increased. Erythropoiesis was strongly hyperplastic with maturation arrest; increase in basophilic normoblasts. Pronounced anisocytosis and microcytosis was observed. The megakaryocytes were remarkably rare. The reticulum was strongly hyperplastic.

Diagnosis: Strongly reactive marrow. No specific characteristics.

November 17, 1950. Tibial punctate: Cell-rich preparation. There was a strong maturation arrest in myelopoiesis down to stem cells. Erythropoiesis was hyperplastic with maturation arrest; increase in basophilic normoblasts and proerythroblasts. Pronounced anisocytosis and polychromasia was observed. The megakaryocytes were normal. The lymphocytes were increased in number without metaplasia. The reticulum was strongly hyperplastic.

Diagnosis: Reactive marrow. No specific characteristics. Systemic disease?

December 20, 1950. Sternal punctate: Cell-rich preparation. There was a strong maturation arrest in myelopoiesis with an increase in myelocytes. A strong maturation disturbance was also observed in erythropoiesis, and anisocytosis was pronounced. The megakaryocytes were normal. The lymphocytes were increased without metaplasia.

Diagnosis: Reactive marrow. No specific characteristics.

January 4, 1951. Sternal punctate: Very cell-rich preparation. There was a maturation arrest in myelopoiesis with an increase of myelocytes and promyelocytes. Erythropoiesis was hyperplastic with maturation arrest, increase in basophilic normoblasts and proerythroblasts. Pronounced anisocytosis. The megakaryocytes were normal. The reticulum was strongly hyperplastic.

Diagnosis: Reactive marrow. No specific characteristics. Systemic disease?

January 10, 1951. Tibial punctate: Cell-rich preparation. There was a strong maturation arrest in myelopoiesis with an increase of myelocytes and promyelocytes. Erythropoiesis was hyperplastic with strong maturation arrest; increase in basophilic normoblasts and proerythroblasts. Pronounced anisocytosis. The megakaryocytes were normal. The lymphocytes were increased without metaplasia. The reticulum was hyperplastic.

Diagnosis: Reactive marrow. No specific characteristics.

X-ray examinations of the skeleton, November 21, 1950: No pathologic structural changes.

Mantoux 1 mg: negative.

Epicritic Summary

A boy fell ill at the age of five months with fever and furuncles on the scalp and neck. He was treated with penicillin and incisions. Shortly afterwards, he again developed a few furuncles which healed spontaneously. At

TABLE IV

Case L1. Blood examinations.

Date	Hemoglobin %	Red blood cells million per mm³	White blood cells per mm³	Stab cells %	Segmented neutrophils %	Eosinophils %	Basophils %	Lymphocytes %	Monocytes %	Unspecified cells %
25/10			15,000	1	3.5	—	—	80	16.5	—
17/11	46	5.18	6,900	6.5	31.5	—	—	52	9.5	0.5
18/11	48									
20/11	47	4.76								
22/11	49	4.82								
24/11	49	4.48	6,100	1	32	0.5	1	53	10.5	2
1/12				—	44.5	2	3	44.5	6	—
2/12				—	39	3	3	47	8	—
6/12				—	36	—	—	57	7	—
9/12				0.5	17.5	0.5	2	77	2.5	—
15/12				1	31	2	1	58	7	—
20/12	46	4.30	8,300	—	23.5	1	1	72	2.5	—
22/12			10,700	—	25.5	5.5	1.5	63	4.5	—
24/12			11,900	—	35	—	1	60	4	—
26/12			6,000	1	18	2	4	70	5	—
28/12			7,900	1	37	5	—	55	2	—
29/12	64	2.90								
2/1			10,400	1	34	1	1	58	5	—
5/1			8,300	—	31	—	2	61	6	—
8/1	75	3.90	6,300	1	24	2	1	70	2	—
10/1			8,200	—	34	1	2	57	6	—
13/1			7,600	—	16.5	1	—	79.5	3	—

the age of nine months, his general state of health was poor. There were no signs of rickets, and the internal organs appeared normal. His hemoglobin was 50 per cent and the white blood cells 15,000 per cu.mm. A differential count disclosed a granulocytopenia with only 4.5 per cent granulated cells. The bone marrow was strongly affected with a significant maturation arrest in myelopoiesis. Erythropoiesis also showed maturation disturbances.

He was observed for two months. His general state of health was relatively good, but the weight increase was poor. He was treated with iron and the hemoglobin rose to 75 per cent. His liver and spleen were never palpable, and there was no enlargement of the lymph nodes. Apart from the initial granulocytopenia, the white blood cells did not show any noteworthy

changes during the period of observation. The bone marrow, on the other hand, showed significant changes the whole time with maturation arrest and maturation disturbances in myelopoiesis as well as erythropoiesis, together with a strongly hyperplastic reticulum.

Concerning the continued course, it is only known that the child was alive and healthy in 1954.

The condition of the bone marrow, and, on one occasion, the blood findings indicate a disturbance of myelopoesis, in principle, similar to that present in granulocytopenia-agranulocytosis. Due to this, and on account of the course of the disease (carbunculosis), it seems likely that this boy suffered from the same disease as the other children reported above, although it took a milder course.

Chapter III

The Affected Children of Group II

Case A1

Boy. Born July 11, 1939. Died August 11, 1939. Cause of death: Pemphigus.

Information given by the mother: The delivery and weight at birth were normal. At the age of two weeks, he fell ill, developing red, boil-like eruptions on the body and fever. He was treated by the district physician, and died at home at the age of 31 days.

Case A3

Girl. Born September 27, 1941. Died October 26, 1941. Cause of death: Carbunculosis.

Information given by the mother and the district nurse: The delivery and weight at birth were normal. The child fell ill at the age of two weeks with multiple boils and fever. She was taken to the district infirmary and was treated there for two weeks. It has not been possible to find her records in the infirmary archives. She died at the infirmary when 28 days old.

Case A5

Boy. Born January 9, 1944. Died February 10, 1944. Cause of death: Pemphigus.

Information given by the mother and the district nurse: The delivery and weight at birth were normal. He fell ill between the age of two and three weeks with fever and boils. The last few days of his life, he was treated in the district infirmary, where he died at the age of 32 days. His records could not be found in the infirmary archives.

Case C1

Boy. Born January 21, 1944. Died February 8, 1944. Cause of death: Phlegmone regionis scrotalis et perinei and sepsis.

The delivery and weight at birth were normal. He was breast-fed. At the age of 15 days he fell ill with swelling and redness of the scrotal region. The following day, he had high fever and a bluish-red miscoloring of the scrotum and penis. He was taken to the surgical ward at Gällivare Hospital. The scrotum was markedly swollen and miscolored and bluish-red. Tem-

perature 40.4° C. Incision was made. The boy died the following day. Autopsy was made. Apart from an edema-like infiltration of the scrotum and the abdomen, nothing abnormal had been observed. However, neither pathologic-anatomic nor blood examinations were made.

Case E1

Girl. Born February 26, 1938. Died March 26, 1938. Cause of death: "Presumably gastric disease".

Information given by the mother, the district nurse, and the district midwife: She was born two weeks before the expected time. Her weight at birth was normal, but she was slightly cyanotic shortly after delivery. She nursed well, and increased normally in weight. At the age of two weeks, she fell ill, developing small furuncles on the body, primarily on the shoulders and the neck. They increased in size. In the neck, a large bluish-red infiltration formed. She developed a cough and high fever. The district nurse saw her one day before death. Her condition was then so poor that the nurse did not think a physician could be of help. She died at the age of 30 days.

Case F2

Girl. Born February 13, 1939. Died March 19, 1939. Cause of death: „Congenital weakness".

Information given by the mother: The delivery and weight at birth were normal. At the age of two weeks, she fell ill with boils. First, a boil developed on the neck, and, later, another one on the right anterior side of the chest. The boil on the neck became so large that the redness and swelling included the whole neck and extended some way down the back. She had fever. She died at home at the age of one month and six days. No physician or nurse saw her during her illness.

Case O3

Girl. Born July 26, 1940. Died August 29, 1940.

Information given by the mother and the district nurse: The weight at birth was 2500 g, and the delivery was normal. She was breast-fed. She was healthy the first week, but developed eruptions at the age of two weeks. These started on the abdomen as small red dots, and became gradually larger until they finally reached the size of about 1 × 1 cm. They were described as furuncles or abscesses. There was no blister formation. She had slight fever, but did not seem ill the first few days. She later became fussy, thinner, and pale. There was no cough or cold, nor was there vomiting or diarrhea. She became progressively weaker, and ate very poorly the last

few days. The urine was then a reddish color. The district physician saw the child and perscribed some ointment. The district nurse had told the physician that she thought that the abscesses looked like „Dirivaara boils" (Dirivaara is the village where family A lives). She died at the age of one month and three days.

Case R1

Boy. Born May 9, 1941. Died June 27, 1941. Cause of death: Thrombophlebitis crur. bilat. cum septicaemia et inanitio.

The weight at birth was 2800 g. He was taken to a doctor on June 18, 1941 for blisters on the legs, and was treated with some ointment. Later, he developed diarrhea and decreased rapidly in weight. The blisters on the legs increased markedly in size, and fever developed. On June 25, 1941, he was taken to the hospital in Kalix. His general state of health was miserable. He was atrophic, dehydrated, and cyanotic. His temperature was 39°C. and the pulse was very rapid. The heart, lungs and abdomen appeared normal. On the inside of the thighs and on the groins, there were large, round sores. Between these, and extending up on the abdomen, there was a hard, red infiltration. He died on the following day. He was then one month and nineteen days old. No histological or blood examinations were made.

Chapter IV

Discussion of the Clinical and Pathological Data

The most important cases for the analysis of the clinical and pathologic-anatomic characteristics of the disease are those in Group I. Of these, cases A9, R4, K2 and L1 have been diagnosed and examined primarily by myself. Cases A9 and L1 have also been studied at the Sachs' Hospital for Children in Stockholm (H. Magnusson, M.D.), and case R4 at the Pediatric University Clinic at Uppsala (Professor B. Vahlquist). In cases D1 and T9, the diagnosis of agranulocytosis was made by physicians who did not know the genetic conditions (M. Sjöholm, M.D., and E. Sahlin, M.D., Gällivare Hospital). Pathologic-anatomic examinations of cases A9, D1, L1 and R4 have been performed by F. Wahlgren, M.D., Stockholm.

The histories of the eight cases in Group II are strikingly similar to cases A9, D1, R4 and T9 in Group I. They all belong to the same family complex as the cases in Group I. The genetic analysis has made it probable that they are all homozygotes in regard to predisposition to the same disease. Because of this, and because of the fact that malignant septic skin infections with lethal outcome are very rare in early infancy, it seems justified to conclude that the children in Group II have had the same disease and died of the same causes as those in Group I.

Two of the children in Group I, cases K2 and L1, differ from the other children in Groups I and II with respect to the clinical data and the course of their disease. In both cases, granulocytopenia could only be observed on a few single occasions. Case K2 lacked septic skin infections, a symptom which characterized all other children in Groups I and II. Both children lived at the time of termination of this investigation and were then 4½ and 5 years old, respectively.

Case K2 was encountered by chance, and has thus not been registered according to the same principles employed in the other cases. For this reason, it has not been included in the genetic analysis.

Clinical Signs and Symptoms

All children have fallen ill at an early age. *The first symptoms of the disease in all cases except one (K2) have been due to infections. Except in case A9 these consisted primarly of septic skin infections.* In case A9, there were

first signs of an infection in the upper respiratory tract with otitis and mastoiditis. Septic infections of the skin started a month later.

The age of onset varied from 1 to 3 weeks in ten of the cases. The others fell ill at the age of 1½, 2½, 3, and 5 months, respectively.

The clinical signs in the initial stage, as well as during the continued course of the disease, were characterized by the appearance of septic skin infections of the type of furuncles, phlegmons, and abscesses in connection with fever. Remarkably enough, no appreciable enlargement of the lymph nodes was present. In addition, the liver and spleen were not noticeably enlarged. *Apart from the septic symptoms and the agranulocytosis, no especially characteristic symptoms of this disease have been observed. All these symptoms initially, as well as during the continued course, can be explained as a consequence of the agranulocytosis and the strongly decreased resistance to infections.*

The most important observation in the peripheral blood was agranulocytosis or grave granulocytopenia which was present in cases A9, D1, R4 and T9.

Case A9, which was observed for over three months, had more than 1000 granulocytes per cu.mm. blood on only one occasion during this period. During the rest of the time, the number of granulocytes varied between 40 and 500 per cu.mm.

Case R4, which was observed for over 2 years, had only on one occasion more than 1000 granulocytes per cu.mm. blood. During the rest of the time, the number of granulocytes varied between 40 and 500 per cu.mm.

Cases D1 and T9 were only observed for a few days before death, and they both had a high degree of granulocytopenia.

Cases K2 and L1 had granulocytopenia only on a few single occasions.

A remarkable finding was the high number of monocytes observed in cases A9 and R4. In both cases, the monocytes, as a rule, made up 50 per cent of the total number of white blood cells. Monocytosis was also observed in case T9. In the two differential counts that could be made, the monocytes made up 16 per cent and 20 per cent, respectively, of the total number of leucocytes.

Agranulocytosis with concurrent monocytosis is not common, but has been described both in adults and children (Thaddae and Bakalos 1939, Tobler and Buser-Pluss 1942, Stodtmeister 1942). Tobler and Buser-Pluss could demonstrate experimentally that the monocytes of their patient had a considerably stronger phagocytic function than those of controls. They believe that this increase in phagocytic function compensated for the loss of granulocytes.

It is possible that in cases A9, R4 and T9 the monocytosis was the result of such a compensatory mechanism. The antibiotic treatment of the infec-

tions in cases A9 and R4 must have been the main cause of the prolongation of life, but, even so, it is likely that an increased resistance towards infections developed in these cases and influenced the course of the disease.

In contrast to the grave changes in the leucocytes, the affected children displayed only moderate and uncharacteristic changes in the red blood cells.

The first observed hemoglobin value in case A9 was 75 per cent. During the first two weeks, this child received blood transfusions three times to improve its general condition. During the following three months, the child did not receive any transfusions. The hemoglobin decreased slowly from 100 to 75 per cent during this period. The number of erythrocytes decreased from 4.7 to 2.8 million per cu.mm. The color index remained close to unity.

Case R4, who had suffered from repeated septic infections for seven months before any blood examination was made, had then 53 per cent hemoglobin and 3.5 million erythrocytes per cu.mm. During the continued course (over two years), the hemoglobin values fell between 51 and 64 per cent, and the number of erythrocytes between 3.1 and 4.0 million per cu.mm.

Case T9, two days before death, had a hemoglobin of 97 per cent and 5.0 million red blood cells per cu.mm. Case D1 had a hemoglobin of 44 per cent and 2.3 million erythrocytes per cu.mm. five days before death.

During the period of observation, case K2 had hemoglobin values between 80 and 95 per cent and a number of erythrocytes between 3.8 and 4.3 million per cu.mm.

Case L1 had hemoglobin values of 46 to 49 per cent with 4.3 to 5.1 million erythrocytes per cu.mm. during the first month of observation. During the following months, the hemoglobin rose to 75 per cent.

A disease of this type characterized by repeated septic infections with fever, must put great demands on erythropoiesis and this should lead to some anemia. The changes in the erythrocytes observed in these children may well be assumed to have developed in this manner, and *the anemias of a moderate degree that have been observed should be regarded as secondary anemias.*

Normal thrombocyte values were observed in cases A9, R4, K2 and L1. Case D1 had a high number of thrombocytes, i.e. 590,000 per cu.mm.

The *bone marrow* was studied repeatedly during the whole period of observation in cases A9, R4, K2 and L1. In cases A9 and R4, *the essential observation was a pronounced maturation arrest in myelopoiesis. The myeloic cell series was completely dominated by myelocytes and promyelocytes. These cell types often appeared in atypical forms with lobed and vacuolated nuclei.* There was an almost complete maturation arrest at the myelocyte state. There was also a considerable hyperplasia of the reticular cells, particularly the lymphoid and monocytoid forms.

In case A9, erythropoiesis was largely intact up to the last few weeks, when signs of maturation arrest and maturation disturbance appeared. In case K4, erythropoiesis showed signs of poor activity the whole time.

In case R4, a number of interesting studies and observations could be made in *culture experiments with the patient's bone marrow.* The results of these *in vitro* experiments suggest that the patient's serum was deficient in such substances as are required for the normal maturation of the bone marrow cells. This was particularly true for myelopoiesis but also for erythropoiesis. An increased maturation of the cells was obtained upon the addition of cysteine to the medium. *Judging from these experiments, it may be assumed that the patient had a deficient ability to utilize the sulphur-containing amino acids which are of importance in cell differentiation.* Because of this, *therapeutic experiments were performed in which cysteine was given to the patient orally and intravenously. No conspicuous improvement could, however, be observed.*

Cases K2 and L1 both showed changes in the bone marrow of, in principle, the same kind. There was found a considerable maturation arrest in myelopoiesis with an increase in myelocytes and promyelocytes and a relative decrease in the more mature forms. However, in none of these cases was the maturation arrest on any occasion as pronounced as in cases A9 and R4. Erythropoiesis was pathological in both cases with maturation arrest and maturation disturbances.

A remarkable finding in cases K2 and L1 was the discrepancy between the bone marrow changes on the one hand and the peripheral blood picture on the other. On account of the changes in the bone marrow, it is surprising that, as a rule, no granulocytopenia was present in the blood.

Of the 14 cases in Groups I and II, 13 have had severe septic infections of the skin manifested as furuncles, phlegmons, abscesses and necrotic sores. Infections of the upper respiratory tract, otitis, mastoiditis and bronchial pneumonia have also been found. *In the 12 cases that died, the infections have been the immediate cause of death.*

In cases A9 and R4, pathogenic strains of Staphylococcus aureus have repeatedly been found in cultures from the skin processes. In addition, growth of α-streptococcus, Bacillus pyocyaneus and coliform bacteria has been obtained in a few samples.

The dominating role of the septic infections is not surprising. In all known agranulocytotic conditions, such infections are common as the immunobiologic protective mechanisms are poorly developed in infants. *However, in case R4 the immunobiologic protective mechanisms in respect to serologic factors were fairly good (antistaphylosin titer 28 units per ml. Gamma globulin 42*

per cent of total serum protein!). This suggests that the infection susceptibility was mostly of cellular origin.

In agranulocytotic conditions in infants described in the literature, suppurative skin afflictions have often been part of the clinical picture.

An important part of the data (Group II) have been collected as histories. The very presence of severe septic skin infections has been considered diagnostic. Thus, no calculations can be made of the frequency of skin infections. There are, however, grounds for assuming it to be very high. It is, however, possible that a number of non-identified cases of the disease could have died from other types of infections before pyogenic skin infections had time to develop.

Therapy

The only therapy available at present is treatment of the infections with antibiotics. Cases K9 and R4 had probably not lived as long as they did without this therapy. It appears probable that the importance of the antibiotic treatment was not limited to counteracting the infections. If the child's life is prolonged with this therapy, conditions are also established allowing the immunobiologic protective mechanisms against infections to develop.

It is possible that the observations made in case R4 on the in vitro maturation conditions of the bone marrow cells, and particularly the role of cysteine, may form the basis of a more causal therapy in the future. This type of therapy would involve an intervention in the maturation of the bone marrow cells, which process would thus be corrected by substitution in one way or another.

Pathology

In all cases, a relatively cell-poor bone marrow was observed containing mainly erythrocyte precursors. The cells of the myeloic system were very poorly represented. *The changes in spleen, liver and lymph nodes were similar, in principle, in all cases. In the spleen,* the follicles were small and lacked germinal centers. *The reticulum was increased* (cases R4 and T9) *and the sinus endothelial cells enlarged* (cases A9 and D1). *In the liver,* there was fattification of the cells (A9, D1, T9) and *enlargement of the Kupffer astral cells* (A9, D1, T9). No phagocytosis was observed, but the Kupffer astral cells contained iron pigment in cases A9 and T9. In the *lymph nodes,* an *enlargement of the sinus endothelium was observed* in cases A9 and T9, and an *increase in the reticulum* in cases R4, T9, and A9 (in biopsy material). An enlargement in the alveolar septae of the lungs was observed in case A9. They were wide and rich in lymphocytoid cells. The reticular cells were enlarged.

It should be pointed out that *no signs of tumor disease, myelogenous or lymphatic leukemia, or aleukemic lymphadenosis could be demonstrated. The signs of an involvement of the reticuloendothelial system which were observed in all cases can hardly be considered as indications of a systemic disease of a reticulosis type. It appears probable that these changes were secondary and an expression of the state of irritation caused by the infection*[1]. Perhaps the lack of leucocytic defense has contributed to these changes.

[1] When this study was almost completed, an interesting discussion of this subject was published by Nordenson (1955).

Chapter V

Different Types of Agranulocytosis

Hematologically, agranulocytosis is characterized by an isolated disappearance of granulocytes from the blood with coincident intact erythropoiesis and thrombopoiesis. Signs and symptoms vary considerably. Angina, necrosis, septic skin processes and icterus may or may not be present. The only constant characteristics of the disease are the changes in the blood and bone marrow.

Agranulocytotic conditions are usually divided into the following types:

1. A form with acute-peracute onset with high fever of septic type, necrotic angina, and strongly affected general state. The disease either quickly leads to death, or else the patient recovers in a relatively short time. This form was first described by Schultz (1922).
2. More slowly developing forms which either lead to death after a relatively long time or slowly turn to complete recovery. These forms are often combined with disturbances in erythropoiesis and thrombopoiesis. The toxic infectious forms belong to this group. They lead over to aleukemia and panmyelophthisis.
3. So-called "Begleitagranulozytosen" where the disappearance of granulocytes is a consequence of a bone marrow disease which destroys, among other things, the myelogenous system (leucosis, tumors, reticulosis).

Etiology

The acute-peracute form of agranulocytosis (the so-called Schultz type) is caused by substances containing a benzene ring. This form has long been considered the result of a violent allergic reaction, an anaphylactic shock towards such substances. The most common allergen is amidopyrine. Other substances such as salvarsan, bismuth, dinitrophenol, gold compounds and sulfonamides, can also play an etiologic role. Their effect is slower than that of amidopyrine and, as a rule, they give rise to the toxic type of agranulocytosis. As a cause of the latter form, infections of an acute or chronic nature have been discussed in the literature.

Pathogenesis

The pathogenesis of the agranulocytotic conditions is incompletely known. Theoretically, agranulocytotic conditions can arise from the following causes:

1. Destruction of the myelogenic tissue.
2. Maturation arrest in myelopoiesis.
3. Disturbances affecting the outflow of the neutrophilic leucocytes from the bone marrow.
4. A peripheral, abnormally high destruction of leucocytes, so-called leucolysis. Such a leucolysis has been difficult to establish, but without assuming its presence, it is, for example, not possible to explain the peracute amidopyrine agranulocytosis. In such cases, it has been shown that the granulocytes can disappear from the peripheral blood in a few hours (Moeschlin 1952).

Among the possibilities just mentioned, maturation arrest has long been considered an essential cause in the origin of agranulocytosis. This maturation arrest can be studied in bone marrow samples. The observations of the morphology of the different cell elements and their relative frequencies form an important basis for the diagnosis of these conditions. They also are very important for following the course of the disease and for determining the prognosis. Two main types of bone marrow changes can be observed:

1. The "classical picture" with a complete depletion of myelopoiesis. Of the cells of the myelogenic series, only a few myelocytes and promyelocytes are present. These often display pathologic forms. At the same time, there is often a considerable increase in the reticular cells, particularly the lymphoid forms and the macrophages, especially the plasmocytous forms.
2. A bone marrow characterized by an enormous cell hyperplasia which especially affects the promyelocytes. More mature granulocytes, on the other hand, are absent or occur in very small numbers only. These changes can most closely be described as a maturation arrest. Fitz-Hugh and Krumbhar (1932) have stressed that this type of bone marrow can be found also in cases with very severe and fatal agranulocytosis.

Between these two main types of bone marrow patterns, there are intermediate forms.

The question of a peripheral destruction of granulocytes as a cause of granulocytopenia and agranulocytosis has received much attention during the last few years. Numerous investigators have demonstrated the existence of specific substances in the blood serum which destroy different cell elements. Thus, Ackroyd (1949) demonstrated a thrombocyte agglutinin in patients with Sedormid purpura. Harrington and co-workers showed, in 1951, that eight individuals with idiopathic thrombocytopenic purpura had a thrombocytopenia-causing substance in the blood associated with the globulin fraction. Evans (1951) demonstrated a thrombocyte agglutinin in

blood of patients with idiopathic thrombocytopenic purpura. He recognized the similarity with hemolytic anemia (cf. Coombs' test) and suggested the name "immuno-thrombocytopenia" for certain cases of thrombocytopenic purpura.

In 1952, Moeschlin and Wagner were able to show that the blood of individuals with amidopyrine agranulocytosis contained a granulocytopenic factor. They could establish that an agglutinin was involved. In later studies (1954), Moeschlin and co-workers have been able to verify their observations further, and show that the agglutinized granulocytes, which disappear from the peripheral blood after agglutination, adhere in the form of leucocyte emboli in the lung capillaries, where they are destroyed.

Moeschlin believes that the different types of bone marrow changes which can arise in agranulocytosis depend on the degree and duration of the peripheral leucocyte destruction and are an expression for the thusly-arisen different degrees of functional stress of the bone marrow. In a first acute stage, the marrow reacts with an attempt to maximum production, resulting in a hyperplasia with a maturation arrest. In later stages, the activity decreases, resulting in a more or less cell-poor bone marrow.

This explanation of the origin of the different bone marrow changes seems reasonable in many cases.

Moeschlin has introduced the concept "*immuno-agranulocytosis*" and believes that, for example, in amidopyrin agranulocytosis, an immunization against amidopyrin has arisen. He also thinks that a number of other agranulocytic and granulocytopenic conditions are due to immunization processes with the formation of antibodies in the form of agglutinins. He divides the agranulocytic conditions according to their pathogeneses as follows:

1. Immuno-agranulocytosis and immuno-leukopenia
    a) Leucocyte agglutinins due to drug sensitization: amidopyrin, butozolidin, hydantoin, sulfonamides.
    b) Leucocyte agglutinins of other immunological origin: primary atypical pneumonia, infectious mononucleosis, virus granulocytosis of cats, cyclic agranulocytosis, transitory granulocytopenia of the newborn, Felty syndrome, lupus erythematodes and other chronic immunogranulocytopenias.
    c) Leucocyte agglutinins of unknown origin.

2. Primary bone marrow damage (usually with pancytopenia)
    a) Due to radiation: X-ray, radium etc.
    b) Toxic: benzol, chloromycetin, urethan, gold etc.

3. Bone marrow obliteration (usually with compensatory extramedullary myelopoiesis):

a) Osteosclerosis
b) Bone marrow infection (Rohr)
c) Neoplastic infiltration:
carcinomatosis, sarcomatosis, myeloma, leukemia.

Chapter VI

Agranulocytosis in Infancy and Childhood

The acute or peracute form of agranulocytosis (of the type described by Schultz) affects, above all, individuals over middle age. Only few cases have been described in children. The most common type of agranulocytosis in childhood is the so-called "Begleitagranulozytose" connected with leukemic conditions. The second most common agranulocytotic conditions belong to the toxic-infectious group. There are relatively few publications on agranulocytosis in children, most being single case reports. Apart from cases of "Begleitagranulozytose", these publications concern benign agranulocytoses caused by different chemicals, most of which belong to currently-used drugs, the prime example being the sulfa group. However, considering the widespread use of these drugs, especially in pediatrics, the frequency of such harmful effects is considerably low. Incidents of toxic agranulocytosis have also been reported where these offending drugs have not been used, or where there have not been signs of other toxic influences. In such cases, the agranulocytosis has been assumed to be caused by the prevailing infection. The agranulocytotic states have been of a relatively short duration, and the children have, as a rule, recovered when the drugs were discontinued, or when the infection was alleviated.

Pronounced granulocytopenia and agranulocytosis of long duration have also been described in children. The concept of *chronic granulocytopenia in children* was coined by Fanconi (1941). Nordmark (1939), Hotz (1941), Salomonsen (1948), Ström (1949), Tobler (1942), Vahlquist and Anjou (1952) and von Vrtilek (1952) have described such conditions.

With regard to the age of onset, the signs and symptoms and the course of disease, two of these cases are of special interest here.

The first case was described by Hotz in 1941, and concerned a twin girl who fell ill at the age of two weeks with fever and furuncles on the legs and abdomen. For a long time she was treated as an outpatient for her recurring septic skin condition. She was taken to a hospital when four months old, and it was found that she had agranulocytosis. An examination of the peripheral blood gave the following information: hemoglobin 57 per cent, erythrocytes 3.82 million per cu.mm., leucocytes 5320 per cu.mm. Differential count: metamyelocytes 2 per cent, stab cells 3 per cent, segmented neutrophils 1 per cent, eosinophils 1 per cent, plasma cells 2 per

cent, lymphocytes 84 per cent and monocytes 7 per cent. Her spleen and superficial lymph nodes could not be felt. Her liver was increased and reached 2 cm below the navel. A bacteriological culture from her abscesses yielded growth of staphylococci.

The bone marrow was markedly hyperplastic. The number of erythroblasts was increased. The most severe disturbance was observed in leucopoiesis, in which immature monocytes and promyelocytes dominated. Transition forms to myeloblasts were also found. *Particularly striking were giant forms of myeloblasts with vacuoles and occasional lobulated nuclei.* The bone marrow displayed signs of a pronounced maturation arrest of myelopoiesis commonly found in agranulocytosis. Consequently, leukemia could be excluded. It was not possible to decide what was the cause of this agranulocytosis, and it did not seem reasonable to assume that furunculosis alone could have given rise to this condition.

Apart from the local treatment of the furuncles and abscesses, prontosil and injections of nucleotrat were given. After this, a temporary increase of the neutrophils was observed. Otherwise, a more or less pronounced agranulocytosis prevailed during the entire period of observation consisting of somewhat more than four months. During this time, the child had pneumonia, as well as recurring furunculosis. When discharged from the hospital, she had 6350 leucocytes per cu.mm., of which only 6 per cent were granulated cells. At a control 2½ years later, she was healthy and her blood was completely normal. She had had no further furuncles. An examination of her twin brother revealed normal blood findings. The microphotographs of the bone marrow of this girl published by Hotz (1941) are very similar to the corresponding microphotographs of my cases A9 and R4. The similarity is particularly striking for the pathological promyelocytes and myelocytes.

The second case was described by Tobler in 1942. This was a girl who, at the age of three weeks, got paronychias and, later, recurring furuncles on the back of her head. At the age of four months, she had an infection with fever, probably influensa, followed by a bilateral otitis media. Her disease was further complicated by furuncles of the neck with extensive skin infiltration. In spite of all these infections, she developed reasonably well. At the age of nine months, she had a lacunar angina with high fever, followed by bronchial pneumonia three weeks later. She was treated with Cibazol for four days, and recovered. At the age of ten months, she again got high fever and angina with considerable swelling of the submaxillary lymph nodes. A few days later, she had a new otitis media, and recovered also this time with Cibazol treatment. An examination of her blood showed agranulocytosis. She had 7250 leucocytes with 3 per cent stab cells, 2 per cent segmented cells, 2 per cent eosinophils, 1 per cent basophils, 67 per

cent lymphocytes, 22 per cent monocytes and 3 per cent plasma cells. The red blood cells were normal and the thrombocytes 940,000 per cu.mm.

The bone marrow displayed a maturation arrest with an increase of immature myeloic cells which was especially pronounced for myelocytes and metamyelocytes. *Marked toxic disturbances, vacuolized nuclei, and atypical forms of nuclei were observed.* She received blood transfusions and pentose nucleotides. After the dissection of a vein for the blood transfusion, she got a wound streptococcal infection with a rash as in scarlet fever.

During the whole course of her disease, she had a more or less pronounced granulocytopenia or agranulocytosis. The monocytes were between 11 and 33 per cent of the total number of leucocytes. She was treated in the hospital for somewhat more than six months. When discharged, she had 8500 leucocytes per cu.mm., of which 2.5 per cent were stab cells, 6 per cent segmented cells (726 granulocytes per cu.mm.) and 13.5 per cent monocytes.

These two cases are, in many respects, similar to those presented in this report. Both of them had recurring septic skin conditions and other infections since infancy. Both displayed agranulocytosis. No specific toxic causes were disclosed. It is true that both of them received sulfa medication to counteract the infections, but, as far as can be judged, with good therapeutic effect. In the case described by Hotz, the agranulocytosis was observed already before these drugs were used and prevailed for four months. Nevertheless, the blood was normal at the control 2½ years later. Both cases were characterized by a prolonged course. In the case described by Tobler in which the agranulocytosis was observed for six months, the continued course is unknown. Both cases displayed a maturation arrest of the bone marrow, which contained numerous pathological types of myelocytes and myeloblasts with vacuolated and lobulated nuclei. By and large, erythropoiesis was normal. One of the cases had a pronounced monocytosis. Concerning the question if these two cases were genetically caused, no answer can be given. They may or may not have been induced by gene difference. The case of Hotz was a twin: but the other one displayed a normal blood pattern. However, they were non-identical twins.

Cyclic Neutropenia

Cyclic neutropenia is a special type of granulocytopenia and agranulocytosis. It was first described by Hale in 1910 and has been studied in detail by Vahlquist (1946) and Reinmann (1949), and it is now recognized as a specific clinical entity.

This disease is characterized by a pronounced neutropenia or agranulocytosis occurring with an interval of about 21 days. The neutropenic phase has a duration of five to eight days, and is usually followed by moderate

fever and local mucous changes of an apthous type in the mouth and throat. Occasionally the infections may be so severe and the agranulocytosis so pronounced that the disease resembles the agranulocytosis of Schultz's type. As soon as the blood becomes normal, the patient recovers promptly. Such periods with neutropenia may recur for several years without undue influence on the general condition of health.

The cause of this disease is unknown. It has been assumed to be congenital. Some twenty cases have been described in literature. In about half of them, the disease was first observed in childhood. It is possible that genetic factors are of importance, but no specific genetic studies have been made. The pathogenesis is also questionable. Moeschlin (1954) believes that this disease belongs to the group of immuno-leukopenias

Transitory Granulocytopenia of the Newborn

Slobodi in 1950 and Lehndorff in 1951 described two siblings with pronounced neonatal granulocytopenia. This granulocytopenia had a duration of 2–3 weeks, after which the blood quickly returned to normal. During the disease, the bone marrow displayed a pronounced maturation arrest of the promyelocyte stage. Lehndorff believes that the granulocytopenia was a toxic allergic phenomenon "and is to be considered as a manifestation of the allergy of the newborn". Moeschlin (1954) believes that this condition belongs to the group of immuno-leukopenias.

Chapter VII

Genetic Factors in Agranulocytosis and Granulocytopenia

In the available literature, I have not been able to find any reports of agranulocytosis with a definite genetic etiology. Several cases with leukopenia in the same family have been reported by Gänsslen (1941), Manzini and Parvis (1947), Hattersley (1947) and Bousser and Nedey (1948).

Gänsslen (1941) reported a pedigree of neutropenia with seven cases distributed over three generations. He claimed this to be a case of dominant inheritance. He did not observe any maturation arrest in the bone marrow. Huber (1939) claimed that certain cases of panmyelophtis should be due to dominant genetic factors. Several cases of eosinophilia in the same family have been reported, among others, by Malmberg (1939). With the exception of these conditions for which the genetic etiology can be accepted only as a suggestion, the only clear-cut genetic condition of the leukocytes is the so-called Pelger nuclear anomaly (cf. Nachtsheim 1941).

Chapter VIII

Genetic Aspects of Infantile Agranulocytosis

by

Jan A. Böök and Rolf Kostmann

Clinical Definition

Infantile agranulocytosis is first described in 1950 by Kostmann who interpreted the disease as a hereditary reticulosis with a monohybrid recessive inhereditance. It is characterized by a more or less complete block in the maturation of the granulocytes. As a consequence, the peripheral blood displays agranulocytosis or a varying degree of granulocytopenia. Resistance against infection is low and affected individuals finally succumb to it, even if life can be somewhat prolonged with antibiotic therapy. Staphylococci infections are most common. Characteristic clinical signs are cutaneous boils and septicemia. Without treatment, the affected infants die within a few months of birth.

Nosography and Demography

The disease was discovered in a North-Swedish geographical isolate. The location of this region is shown in Fig. 1 (page 8). The area is 1073 sq. miles and, in 1950, the population totalled 9215. It is a typical farming and lumber district and, since 1644, has been a separate parish, Överkalix. Apart from the geographical location, the relative isolation is due to scarcity of roads and lack of railways.

The demographic features of this region have been similar to those of the isolates investigated by Sjögren (1932 and 1935) and Böök (1953). From 1900 to 1950, the population increased from 5539 to 9215 (cf. Table V).

The crude birth rates, although decreasing during the last 10 years, have been considerably higher than the average for Sweden (cf. Table VI).

Up to 1940, the general mobility of the population, as measured by crude migration rates, was relatively low (cf. Fig. 14, p. 66).

In conclusion, it can be stated that the demographic and geographic characteristics of the investigated area justify its classification as an isolate in terms of population genetics.

TABLE V

Parish of Överkalix. Population 1900–50.

Year	Total population		
	Males	Females	Total
1900	2,862	2,671	5,539
10	3,135	2,878	6,013
20	3,433	3,084	6,517
30	3,835	3,561	7,396
40	4,463	4,101	8,564
50	4,800	4,415	9,215

TABLE VI

Crude birth rates per 1,000 population.

Year	Parish of Överkalix	County of Norrbotten	Sweden
1931/35	29.2	22.9	14.1
1936/40	28.7	22.7	14.8
1941/45	31.1	25.7	18.7
1946/50	25.7	23.6	18.2

It should, of course, be observed that "isolate" is a *relative* concept referring to a group of people for whom matings within the group are significantly commoner than matings outside the group. Full discussions have been given by Wahlund (1928) and Dahlberg (1938 and 1947).

Collection of Data

The original family found by Kostmann was family A of the major pedigree (cf. Fig. 15, p. 67). The parents were third cousins. Starting from the common ancestors of these parents, all descendants were registered. A preliminary pedigree was drawn up which contained all sibships in which at least one child had died of unknown cause during infancy.

From the observations on family A which contained the cases A1, A3, A5 and A9, it was decided what signs and symptoms the registration of further *propositi* had to be based on.

The following Definition of a Propositus was agreed upon: *a child who had died during infancy displaying cutaneous boils and septicemia with or without confirming blood and bone marrow pathology, or still living and displaying the same symptoms and confirming pathology.*

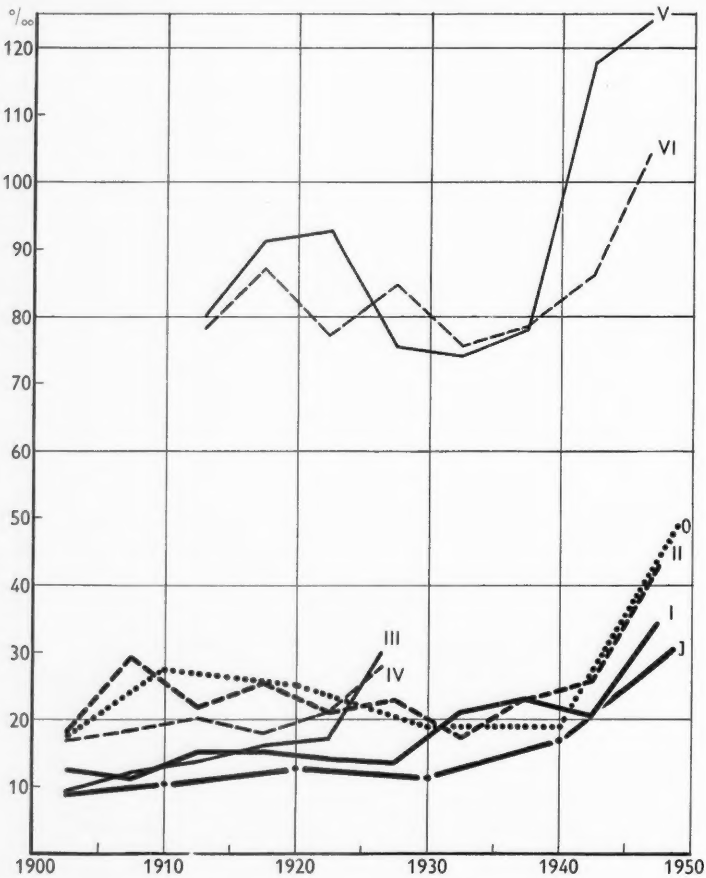

Fig. 14. Crude migration rates per 1000. I and II, in- and outmigrations, respectively, for the North-Swedish isolates investigated by Böök (1953). III and IV, in- and outmigrations, respectively, for the North-Swedish isolates investigated by Sjögren (1932 and 1935). V and VI, in- and outmigrations, respectively, for the cities of Norrbotten county. J and O, in- and outmigrations, respectively, for the parish of Överkalix.

As sufficient information could be obtained only for the last generation, the revised pedigree became considerably smaller. At this stage, it contained the families A, D, E, F and L with a total of 8 affected children. The first of these children was born in 1931.

It was then decided to make a complete registration of all children with infantile agranulocytosis born from 1931 to 1953, inclusively. For this

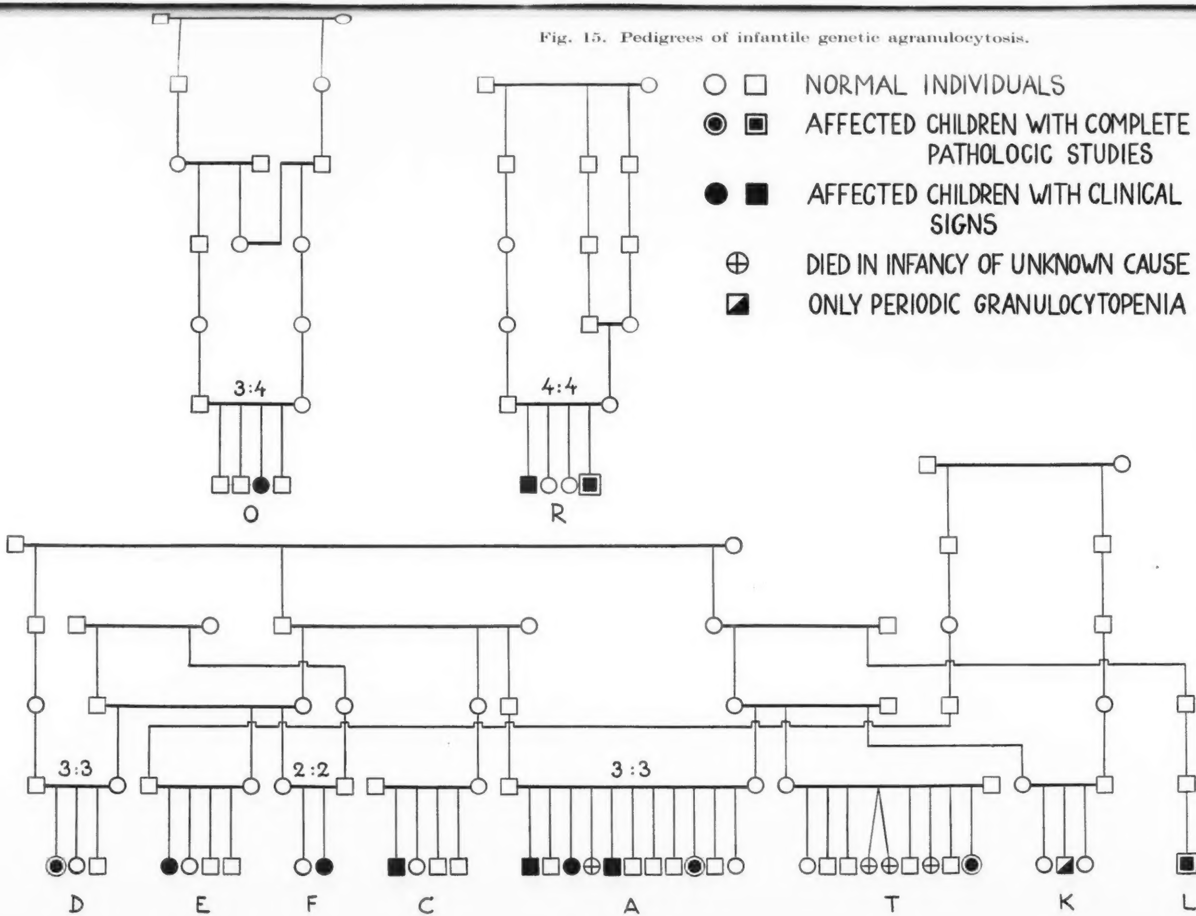

Fig. 15. Pedigrees of infantile genetic agranulocytosis.

purpose, the death certificates for all children who had died before the age of one year were scrutinized for this period. For every case where the cause of death was in doubt, further information was obtained by interviewing the parents, the local physician and the district nurses. This screening procedure gave only one new addition, O3, to the already-known cases.

While this work was in progress, three additional cases were reported by physicians, two from the parish of Gällivare and one from the parish of Nederkalix, all in the same county but outside the investigation area. The first two of these cases, C1 and T9, were shown to belong to the major pedigree. In both families, the mothers were born in the investigation area and the fathers had ancestors there. Family R came from Nederkalix, which is a neighbouring parish.

Consequently, all the cases so far known have been derived from a fairly circumscribed geographical area.

With reference to the definition of *propositi* given above, we are satisfied that the registration within the investigation area during 1931–1953 was complete. All genealogic data have been obtained through parish registers.

Genetic Analysis of Family Data

We are concerned with a lethal condition which showed accumulation in one major pedigree, and which was repeated in two sibships. Therefore, to begin with, the hypothesis of a single recessive gene difference should be tested.

In the pedigrees (Fig. 15), family K has been excluded from the genetic analysis because the child K2 was not ascertained to be a *propositus* according to definition. This child displayed only a moderate granulocytopenia on a few occasions and had no cutaneous infections or septicemia. It is of course possible that this case as well as case L1 is a heterozygote. This left nine families with a total of thirteen affected children. In all families, the parents and siblings have been subjected to blood studies. Bone marrow studies were made in four parents. However pathologic changes were found in none of them. There is thus so far a clear-cut difference between affected and nonaffected.

Consanguinity

In five of the nine families, the parents were related, in one as 2:2, in two as 3:3, in one as 3:4 and in one as 4:4. No data on consanguinity rates are available for this particular region. However, there is no reason to assume that the mean coefficient of inbreeding (α) should differ appreciably from another region of similar type in the same county investigated by Böök

(1956), who estimated $\alpha \geq 0.002$. This was calculated on consanguinities 1:2, 2:2, 2:3, 3:3, 3:4, 4:4 4:5 and 5:5. If we calculate the mean inbreeding coefficient (α) for the nine families according to the equation where r_i is

$$\alpha = \frac{\sum_i (r_i n_i)}{\sum_i (n_i)}$$

the degree of relationship and n_i the number in the i-th class, we obtain α = 0.012. This should be about 6 times higher than expected for random families.

Furthermore, *the fact that seven of the families could be referred to a common ancestral pair strongly indicates a recessive type of inheritance.*

Test of simple recessive inheritance

The data have been obtained partly as a complete ascertainment of all cases within the population of the investigation area during 1931–1953, and partly through affected individuals born outside this area but genetically derived from it. All affected children except R1 are *propositi*.

In Table VII (p. 70), a test of simple recessive inheritance has been made according to the *a priori method*, devised by Apert and Bernstein (cf. Bernstein 1929 and Macklin 1938). The *observed and expected figures are in good agreement.* Strictly, however, it is not quite correct to include the families C, L and R, as they do not belong to the complete ascertainment. On the other hand, as shown in table VIII (p. 70, the exclusion of these families is immaterial. We obtain a difference of 0.24 between observed and expected affected children, which is less than one standard deviation (1.74).

In the major pedigree, there are four children, including one pair of twins of unknown zygosity, who died in infancy. Although the occurrence of signs typical of infantile agranulocytosis in these children was denied, there is always the possibility that such signs were overlooked. However, the evidence of a simple recessive inheritance as presented here seems sufficient to make superfluous the tentative inclusion of all or some of these cases in the calculations.

In summary, the genetic analysis of the family data has lead to the conclusion that infantile agranulocytosis, as observed in this North-Swedish population, is basically due to a single recessive gene difference. Consequently, we suggest that this new disease entity should be called INFANTILE GENETIC AGRANULOCYTOSIS.

Population Genetics

The detection of this new recessive lethal in man represents another example of the accumulation of a mutation in a particular isolate. New recessive mutations arise continuously, but their chances of survival depend

Table VII

Infantile genetic agranulocytosis. Test of simple recessive inheritance. *A priori method*. As affected have been included all individuals with complete pathologic studies and those with typical clinical manifestations of cutaneous infections.

Sibship size	Total no. of sibs	No. of affected children		No. of normal children		χ^2 $\Sigma (o-c)^2/c$
		o	c	o	c	
2– 4	21	7	8.3	14	12.7	0.337
9–11	20	5	5.3	15	14.7	0.023
Totals	41	12	13.6	29	27.4	0.360

$DF = 2;\ 0.9 > P > 0.8$

Table VIII

Test of simple recessive inheritance. A priori method. The calculations have been made on families A, D, E, F and O, representing complete ascertainment within the investigation area.

Family size	No. of families	No. of affected observed	No. of affected expected	Variance
2	1	1	1.1428	0.122
3	1	1	1.2973	0.263
4	2	2	2.9258	0.840
11	1	4	2.8710	1.805
Totals	5	8	8.2369	3.3030

$$\sigma = \sqrt{3.03} = 1.74$$

on such factors as genetic drift and breeding structure. Their appearance in homozygous form in a particular pocket of the population is rather a matter of chance, unless the heterozygotes display a positive selective value (increased fitness). It means that once a mutation has occurred in a particular individual, by far the largest probability is that it will die out after a few generations without having established itself in homozygous condition. Such mutations will, of course, pass without being noticed. However, it may happen, as in this case, that, within a specific population, the heterozygotes accumulate, and the gene reaches a frequency which is high enough to be a safeguard against its rapid eradication between one or two generations. It will, of course, still be subjected to considerable genetic drift. The chances that homozygotes appear in the population depend furthermore on the amount of inbreeding. Inbreeding as such does not affect the

accumulation of a recessive lethal gene, but will increase the chances for homozygotes to appear more rapidly after the original mutation. Once the eruption of homozygotes has started, which means that heterozygotes have become fairly common, continued inbreeding will tend to decrease the gene frequency. If the population is large enough, a state of balance between selection and mutation will be reached where the gene frequency remains fairly constant from generation to generation. Practically all rural populations, however, are rather small and chance fluctuations in gene frequencies will continue.

The expressions "large" and "small" populations refer here to the effective population size, i.e. the number of individuals who actually reproduce and transfer their genes to the next generation. This number is, of course, considerably smaller than the total number of individuals at any given time.

Böök (1956) suggested an estimate of the effective population size in geographic isolates based on the number of married women aged 15–45 at intervals of approximately one generation (m_1, m_2 ... m_i) as follows:

$$\frac{1}{N} = \frac{f}{n}\left[\frac{1}{2m_1} + \frac{1}{2m_2} + \cdots \frac{1}{2m_i}\right]$$

where N is the mean effective population size and f the frequency of fertile marriages.

In this population, data on m were available only from 1910. Taking the years 1910 and 1950 as $m_1 = 473$ and $m_2 = 958$ and f as 0.95, we obtain $N = 1204$. Before 1910, the m-values must have been much smaller, indicating that the estimate of N as referring to the period when the new mutation spread in the population is too large. Still, in terms of population genetics, an average effective population size of about 1200 is quite small and subjected to pronounced genetic drift.

Calculations of gene frequencies are based on the assumption that the population is in equilibrium. For a small population like the present one, such an assumption is only approximately true. Therefore, only crude estimates of the distribution of this lethal recessive gene can be given.

During 1931–1953, a total of 5390 children were born within the investigation area. Of these, 8 were affected, i.e. 1/674 or 0.0015. This figure then equals the general morbid risk.

The gene frequency should be $\sqrt{0.0015}$*, or approximately 4 per cent, and the incidence of heterozygotes about 8 per cent.*

Biochemical Genetics

The most important general implications of this investigation are connected with the biochemical findings.

In an earlier paper Böök and Kostmann (1956), described an observation orginally made by Vahlquist and Plum (1952)[1] on the addition of cysteine to bone marrow cultures *in vitro* being able to normalize the maturation of the granulocytes. Otherwise, a blockage of the myelopoesis at the stage of the myelocyte was observed. So far it has been possible to study only one case (R4).

These findings suggest that the gene for infantile agranulocytosis in its biochemical action has some effect on cysteine metabolism.

The combination of genetics, biochemistry and cell culture work appears promising. This approach will be followed up, as it has the obvious advantage of bringing medical genetics within reach of experimentation. As a method of detecting metabolic deviations in genetic diseases on the level of cellular biochemistry, it should be able to give at least suggestive results. Although we know that the action of genes are more or less directly concerned with metabolic pathways, there have so far been few starting points for human biochemical genetics. It seems possible that the above suggestion might overcome some of these difficulties.

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[1] Personal communication (see also p. 34).

Chapter IX

Comments

The details of the clinical and pathological studies justify the conclusion that all affected children are affected by the same disease, the most characteristic feature of which is agranulocytosis. The genetic studies show, beyond any doubt, that this disease *primarily* is genetically determined, and is caused by a single recessive gene difference.

The question of the *pathogenesis* of this disease is of considerable interest. At the present time, no more than different possibilities can be discussed. The lethal gene, in homozygous constellation, may influence the pathogenesis in a number of different ways.

Firstly, it may cause a primary insufficiency of the myeloic system, i.e. a blockage of cell maturation. In such an event, the children should display agranulocytosis already at birth. However, there is no proof that this was the case because so far it has not been possible to make any blood studies immediately after birth. Child T9 had no agranulocytosis three weeks before the onset of the disease which led to her registration for this investigation. While undergoing treatment for furunculosis and abscesses, she had 8100 white cells per cu.mm. with 17 per cent stab cells and 8 per cent polymorphonuclear granulocytes. Consequently, there is only evidence that the agranulocytosis appears very early, usually during the first few months. It is, of course, possible that the disease is congenital.

Secondly, the gene effect could be to decrease the resistance against infections. As a consequence, any slight infection would take a serious course and put such a stress on the bone marrow that immediate insufficiency would result and that the individual would develop agranulocytosis. This agranulocytosis would then lead to a further decrease of resistance against infection, with increased stress on the bone marrow, and so on.

Thirdly, the gene effect could lead to an autoagglutination of the granulocytes followed by their rapid destruction in the peripheral blood, and, finally, to an insufficiency of the bone marrow similar to that which happens in the immuno-agranulocytosis described by Moeschlin.

The observations during this investigation do not admit definite conclusions. However, *the most likely explanation is that the genes cause a primary insufficiency of the bone marrow*. Especially the cells of the myeloic system appear to be affected. It is possible that this weakness is present in some

cases already at birth, or shortly thereafter. *Perhaps, in most of the cases, the bone marrow becomes definitely insufficient only when infections cause a stress upon it — or perhaps first after the end of action of a hypothetical diaplacental factor. When agranulocytosis has appeared, the disease will lead to death because of a vicious circle caused by the simultaneous interaction of agranulocytosis and infection.*

The above explanation seems justified on the basis of the following facts:

(1) The cell culture experiments with bone marrow from case R4. These indicate that the normal maturation of the bone marrow cells is disturbed and that this disturbance may depend on an insufficient utilization of certain substances in the blood—notably the sulphur-containing amino acids—which appear to be of importance for cell differentiation.

This exemplifies the fact that any genetic factor basically must influence biochemical pathways.

(2) The children K2 and L1 both had a pathologic bone marrow during a long time of observation. By and large, the changes were of the same type as in the other cases although the bone marrow was more hyperplastic and showed no actual blockage but rather a considerable reduction of the number of polynuclear cells. It seems especially remarkable that, in these children, the granulocytes of the peripheral blood were — with a few temporary exceptions — normal in number and displayed no signs of a shift to the left.

General Summary

1. A new disease of infancy is reported. It has been concluded that this disease constitutes a clinical as well as a genetical entity, and it has been named INFANTILE GENETIC AGRANULOCYTOSIS.

2. The main characteristics of this disease are as follows. The onset occurs during early infancy with fever and skin infections manifested as boils and phlegmons. There is a complete or almost complete lack of granulocytes in the peripheral blood. The preparations from bone marrow biopsy or autopsy display a marked retardation or block in the maturation of the myelopoietic cells. Without treatment, the disease runs a short course with lethal outcome. If the infections are treated with antibiotics, the affected children may survive for several months or even years. However, the agranulocytosis and the pathologic changes of the bone marrow remain, and the lethal outcome can only be postponed.

3. Fourteen children (boys and girls) with this disease, belonging to nine families, are described. For six of them detailed clinical and histological data are reported. The remainder had died before the start of this investigation, and only catamnestic data were available. Two of the six children mentioned above displayed only a temporary agranulocytosis, but the pathological changes of the bone marrow resembled those of the other four children.

4. Most of the affected children could be referred to a common pedigree by objective genealogic methods. All of them belonged to the same geographical isolate inasmuch as the children were either born there or both parents had ancestors there. Close consanguinity between the parents was established in five of the nine families.

5. The genetical analysis supports the conclusion that this disease primarily is caused by a single recessive autosomal gene difference (a simple recessive mutation).

6. The geographical isolate which was surveyed had a population of 9235 in 1950. The incidence of infantile genetic agranulocytosis among newborns was estimated at 1 in 674 or 0.0015. This should correspond to a gene frequency of approximately 4 per cent and heterozygote frequency of approximately 8 per cent. These latter two estimates, however, are somewhat uncertain due to a small effective population size.

7. Pathogenetically, infantile genetic agranulocytosis is probably caused by primary changes in the function of the bone marrow. Certain results of experiments with *in vitro* cultures of bone marrow cells seem to indicate a primary effect of the mutated gene on certain metabolic pathways, possibly of the amino acids.

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Morphology of the Pancreas and Glucose Tolerance in Biliary Fistula, in Common Bile Duct Obstruction and After Ligation of Pancreatic Duct

By

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Almqvist & Wiksells Boktryckeri AB UPPSALA

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FROM THE PAEDIATRIC CLINIC OF KAROLINSKA INSTITUTET AT KRONPRINSESSAN LOVISAS BARNSJUKHUS (HEAD: PROFESSOR CURT GYLLENSWÄRD), STOCKHOLM, SWEDEN AND THE INSTITUTE OF PATHOLOGY (HEAD: PROFESSOR GÖSTA T. HULTQUIST), UNIVERSITY OF UPPSALA, SWEDEN

Morphology of the Pancreas and Glucose Tolerance in Biliary Fistula, in Common Bile Duct Obstruction and After Ligation of Pancreatic Duct

EXPERIMENTAL OBSERVATIONS IN NON-DIABETIC AND ALLOXAN-DIABETIC RABBITS

by

YNGVE LARSSON

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Translated from the Swedish

by

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Victor Pettersons Bokindustri Aktiebolag
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PREFACE

The present investigation was carried out at the research laboratory of the Paediatric Clinic, Kronprinsessan Lovisas Barnsjukhus, during the years 1953 to 1955.

My Chief, Professor Curt Gyllenswärd, followed my work with the greatest interest ever since the beginning. I am glad to have this opportunity of acknowledging my sincere debt of gratitude to him for his encouragement, for many stimulating discussions, and for the boundless generosity with which he placed all the resources of the Clinic at my disposal.

Professor Gösta T. Hultquist followed the course of the investigation from its initial stages until its completion. The preliminary experiments were performed under his direct supervision at the Institute of Pathology of the Medical School, Gothenburg. Professor Hultquist's wide experience of experimental diabetes research and his great knowledge of the relevant fields have been an invaluable asset. I am deeply grateful for his kindness, interest and many helpful suggestions.

Certain parts of the investigation were carried out at Chemistry Department II, Karolinska Institutet, with the kind permission of Professor Erik Jorpes. For this courtesy, and for the interest which Professor Jorpes showed in my work, I offer him my sincere thanks.

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Laborator Gunnar Bergman, of the Department of Dental Histopathology, the State Dental Institute, Stockholm, kindly placed certain apparatus at my disposal. For this, I wish to offer him my warm thanks.

The investigation would have been impossible without the continuous assistance of many persons. I take this opportunity of thanking:

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Miss Gurli Westlund, Apoteksvarucentralen Vitrum, who with unfailing accuracy made all the blood sugar determinations.

For valuable co-operation in other respects, my thanks are due to Mrs. Anna-Greta Arwén, Dr. Gunnar Christiansson, Laboratory Nurse Birgit Czar, Miss Karin Eriksson, Head Nurse Svea Hagman, Miss Maggie Karsten, Mrs. Brigitte Paulson, Med. kand. Harald Sanne, Mrs. Maj Söderlund, as well as to my colleagues and the Staff of Kronprinsessan Lovisas Barnsjukhus.

Fil. lic. Nils Blomqvist of the Statistical Research Group of the University of Stockholm assisted me with the statistical analyses of the material. I am greatly indebted to him for numerous discussions and for valuable advice.

All the diagrams were drawn by Mr. Anders Henschen. His ability has been of the utmost help.

Mrs. Erica Odelberg translated the paper. Her experience, profound knowledge of medical writing, skill and indefatigability have been a great asset.

My wife, Gerd Larsson, assisted me in all the operations, performed a large proportion of the urinalyses, did much of the planimetry and continuous secretarial work. Her unselfish co-operation and constant encouragement are inestimable.

The expenses of the investigation were defrayed by generous grants from Karolinska Institutets Medicinska Forskningsanslag, Nordisk Insulinfond, Svenska Livförsäkringsbolags Medicinska Forskningsnämnd and Svenska Läkaresällskapet.

Stockholm, March 1956.

Yngve Larsson

INTRODUCTION

In a study of the effect of experimental biliary fistula on the endocrine organs in the dog, LERICHE & JUNG (1939) found changes in the islets of Langerhans, consisting of an increase in their number, volume and vascularity. These observations could be complemented clinically in a woman with severe diabetes, in whom the bile had been drained off for about a year through a fistula opening from the gallbladder. During this time, the patient—who had earlier required 80 to 100 units of insulin a day—was able to dispense with insulin and still have practically normal blood sugar and sugar-free urine. When the fistula was closed, the diabetic symptoms reappeared after some time, with the same severity as before.

A few years later, VERNE reported similar observations, both experimentally in dogs, and in a non-diabetic child. The latter had a tumour compressing the common duct, and a biliary fistula had been created as a palliative measure during the last three weeks of his life.

(The papers of LERICHE & JUNG and of VERNE will be reviewed in more detail in Chapter 1.)

It was these observations that led me to make the present investigation. If it could be confirmed that the islet tissue of the pancreas was under the influence of the bile flow to the intestine, I considered that such a mechanism might be of clinical importance, in addition to its theoretical interest. It was, however, evident that the aforementioned investigations needed to be supplemented from several viewpoints. For example, no quantitative method had been used for estimation of the size of the islet tissue, and control material comparable from the nutritional standpoint was lacking. I therefore found it desirable to perform such investigations with due consideration paid to these factors.

When a systematic quantitative analysis of the islet tissue is to be made, it is necessary to choose such a small experimental animal that examination of a relatively large number of animals is practicable. For this reason, the dog is unsuitable. On the other hand, creation of a biliary fistula requires an animal sufficiently large for the intervention to be technically feasible. Moreover, it must be an animal that can be kept alive with a functioning biliary fistula for a sufficient time for any changes in

the pancreas to take place, *i. e.*, for about three to four weeks. I found the rabbit to be the animal best fulfilling all these criteria.

In addition to the morphologic analysis, I considered that it would be of interest to study the glucose tolerance in biliary fistula animals. This was because LERICHE & JUNG had found signs of increased glucose tolerance and a tendency to hypoglycaemia in their animals. For this purpose as well, the rabbit is a suitable experimental animal.

Alloxan, which is such a valuable tool in experimental diabetes research, was not yet available when the investigations cited were made. There was therefore reason to complement my investigations on non-diabetic animals with a study of the effect of biliary fistula in alloxan-diabetic animals.

Thus, the primary object of the investigation was:

1. *By means of a systematic quantitative analysis, to investigate the effect of biliary fistula on the glucose tolerance and the islet tissue of the pancreas in non-diabetic and alloxan-diabetic rabbits, as compared to non-operated but otherwise similarly treated non-diabetic and diabetic controls.*

When experiments were made according to this scheme, I was able to make the following observations at a relatively early stage. Firstly, the increase in size of the islet tissue which had been found by the aforementioned authors also seemed to appear in the rabbit pancreas. Secondly, the biliary fistula intervention also produced a generalized enlargement of the *whole* pancreas. Moreover, when the operation was unsuccessful for technical reasons, and only closure of the common duct with resulting *biliary stasis* was produced, changes appeared in the pancreas of the same type as in the biliary fistula experiments. I therefore considered it advisable to extend the investigation as follows:

2. *To investigate, with the same technique, the effect of biliary obstruction on the glucose tolerance and the pancreatic tissue in non-diabetic and alloxan-diabetic rabbits, as compared to non-operated, non-diabetic and diabetic controls.*

The results of these experiments indicated that, in the rabbit, the absence of bile in the intestine leads to an increase in the size of both the exocrine and endocrine parenchyma of the pancreas. It is true that the results did not answer the question whether one and the same mechanism or possibly separate mechanisms are responsible for these changes. I nevertheless thought it conceivable, in view of the common embryology of the pancreatic tissues, that a stimulus to exocrine growth could produce an increase in the size of the islet tissue as a secondary result.

Consequently, I felt it of interest to study the course of events in the islet tissue when the exocrine parenchyma atrophies, as is the case after

ligation of the pancreatic duct. Although this intervention has been the object of numerous investigations ever since the end of the last century, opinions differ as to the fate of the islets in this condition. Most authors contend that the islet tissue remains unchanged; others consider that the islets atrophy in similarity to the exocrine parenchyma, whereas still others have described varying degrees of hypertrophy of the insular apparatus. However, as far as I have been able to ascertain, no workers have made systematic quantitative studies of the islets after ligation of the duct. I therefore enlarged the scope of the investigation as follows:

3. *To investigate, with the same quantitative technique, the glucose tolerance and the size of the islet tissue in the atrophic pancreas after ligation of the pancreatic duct, as compared to the conditions in rabbits with functioning exocrine parenchyma.*

The last-mentioned question is of particular interest against the background of the investigations reported by WALPOLE & INNES, who found rabbits to be resistant to the diabetogenic effect of alloxan after ligation of the pancreatic duct. This phenomenon has, it is true, been stated by FERNER to be only apparent. In his opinion, cessation of exocrine pancreatic function leads to such severe liver damage that diabetic symptoms such as hyperglycaemia and glycosuria are as inconceivable as in the combination of hepatectomy and diabetes. If FERNER's hypothesis—which does not seem to be based on any personal investigations—were correct, morphologic analysis of the pancreas from animals given alloxan *after* ligation of the duct should give similar results to those obtained in animals in which diabetes has been produced by alloxan *before* ligation of the duct. Moreover, there would be reason to expect that such alloxan-diabetic animals, in which the pancreatic duct had been ligated secondarily, would exhibit gradual disappearance of hyperglycaemia and glycosuria in connexion with onset of the supposed liver damage. These considerations formed the basis of the fourth problem:

4. (a) *To investigate whether, after ligation of the pancreatic duct, rabbits are resistant to the diabetogenic effect of alloxan, as stated by* WALPOLE & INNES.

(b) *To make a quantitative analysis of the glucose tolerance and the islet tissue in such animals, as compared to definitely alloxan-diabetic animals in which the pancreatic duct had been ligated secondarily.*

(c) *To investigate whether the diabetic condition in animals of the latter type undergoes any systematic change in connexion with cessation of the exocrine pancreatic function.*

Histologic and histochemical examination of the liver in these types of

animals, as well as in animals with the pancreatic duct ligated but not given alloxan, would also answer the question whether liver damage of the severity postulated by FERNER actually occurs.

Hyperplasia of the islet tissue has been reported by several workers after *glucose administration*. However, these investigations as well have been performed in most cases without the use of quantitative micromorphologic technique. As a complement to the investigations already listed, I considered it of interest to ascertain whether such hyperplasia could be observed with the quantitative technique used. If this proved to be the case, a comparison could then be made between this increase in size and that produced by the biliary fistula and biliary stasis experiments. Finally, my intention was to investigate whether any change occurred in the diabetogenic effect of alloxan when it was given after several weeks' glucose administration. The fifth problem could therefore be formulated as follows:

5. *To investigate the effect of long-term parenteral administration of glucose on the glucose tolerance and the size of the islet tissue, as well as the effect of alloxan on animals thus treated.*

The present paper comprises a report of the attempts made to answer the problems listed in the aforegoing.

A brief survey is given in Chapter 1 of some earlier investigations of interest in the present connexion. Since in many respects the problems listed have extensive pathophysiologic implications, I have been unable to present a complete review of the literature. Nor has it been possible to give an account of the numerous publications dealing with various aspects of alloxan diabetes. Many of the problems in the present investigation have, however, been discussed in a number of monographs and reviews, to which reference is made (ALLEN 1913, BABKIN 1950, BARGMANN 1939, BENSLEY 1914/1915, BEST *et al.* 1954, DE MOOR 1953, DUFF 1945, FERNER 1952, GOMORI 1943, HAUGAARD & MARSH 1953, JOSLIN *et al.* 1952, KORP & LECOMPTE 1955, LAGERLÖF 1942, LAZAROW 1949, LICHTMAN 1953, LUKENS 1948, MANN 1927, SOSKIN & LEVINE 1946, STEINCKE 1951, TEJNING 1947, WARREN & LECOMPTE 1952, ZIMMERMANN 1952).

A discussion of several of the methods used was found to be necessary. In order to avoid making the account of the methods in Chapter 3 unwieldy, this discussion has been assembled in a special chapter (Chapter 4). A brief description of earlier investigations on the evaluation of glucose tolerance tests, biliary fistula technique and methods for determination of the size of the islet tissue is also given in this chapter.

Many of the results of the present investigation are given in the form of tables. The results in the individual animals are collected at the end of the paper. Tables I: 1 to I: 12 show the results of the glucose tolerance tests, Tables II: 1 to II: 15 the body size and the pancreas size, Tables III: 1 to III: 15 the results of analyses of the islet tissue, and Tables IV: 1 to IV: 15 the results of the alpha and beta cell counts.

ABBREVIATIONS

R	rabbit (rabbits)
BW	body weight (kg)
BS	body surface (dm^2)
mg-min	milligram-minutes (glucose tolerance area)
D	duodenal part of pancreas
L	splenic (lienal) part of pancreas
DL	D+L
P	total magnified parenchymal area in a section of pancreas (mm^2)
p	real parenchymal area in a section of pancreas (mm^2)
V_p	total parenchymal volume of pancreas (mm^3)
V_p/BW	parenchymal volume of pancreas per kg of body weight (mm^3)
l	number of sections from one animal at intervals of 1,200 μ
k	number of sections from one animal in which a sample of islet area was measured
I	total measured magnified islet area in a section of pancreas (mm^2)
N	number of islets measured in I
M	mean islet area of islets N (mm^2)
n_i	total number of islets counted in one section
I_c	total calculated magnified islet area in one section (mm^2)
i	real islet area corresponding to $S(I_c)$, (mm^2)
V_i	total islet volume of pancreas (mm^3)
V_i/BW	islet volume of pancreas per kg of body weight (mm^3)
m_i	mean islet area in one animal (μ^2)
n_i/BW	total number of counted islets in one animal per kg of body weight
n_c	calculated total number of islets in the whole pancreas in one animal
N.A.D.	nothing abnormal detected
u	units of insulin

For statistical symbols, see pages 56—58.

CHAPTER 1

SURVEY OF THE LITERATURE

THE LIVER AND DIABETES

"In hypotheses concerning the etiology and mechanism of diabetes, from the earliest times to the present, the liver has generally played a prominent part. Its importance in general metabolic processes, and as a storehouse of glycogen, and as a regulator of the sugar of the blood, have naturally pointed toward it in connection with a disease in which metabolic processes and the economy of glycogen and sugar are so markedly disturbed." (ALLEN 1913).

The central role of the liver in the mechanisms of blood sugar regulation and carbohydrate metabolism has been particularly well established by the fundamental work done by MANN, and later by the investigations of SOSKIN and his co-workers. Despite this fact, it is improbable that the liver has any *specific aetiologic* importance in diabetes (ALLEN; WARREN & LECOMPTE).

Gross lesions of the liver are not especially common in diabetes. *Tests of the liver function* in this disease have been carried out by LEEVY *et al.*, among many others. They made "a battery" of different liver function tests in a series of 380 diabetic patients. Abnormal results in two or more of these tests were obtained in 148 (38.9 per cent), this being interpreted as a sign of liver dysfunction. Such dysfunction was found to be more common in patients with pronounced dietary and insulin deficiencies. Specific therapy against the liver disorder was applied in 65 cases. The results indicated that in 15 cases the hyperglycaemia was secondary to the liver dysfunction, but that in 48 the liver damage was secondary to the diabetic condition, whereas in 2 cases coincidence was present. In 47.8 per cent of the cases, treatment of the liver dysfunction led to a decreased insulin requirement.

The relatively high incidence of pathologic liver function tests present in the series of LEEVY *et al.* could not be confirmed by MARBLE, who found the results of such tests in patients with well-controlled diabetes without

complications to be the same as in a control group of non-diabetic individuals.

In the experience of TAUB *et al.*, based on the results of liver function tests including the glucose tolerance test, a not inconsiderable number of adult diabetics do not have diabetes in the ordinary sense, but their hyperglycaemia is caused by "low grade liver damage", *i. e.*, a liver dysfunction hyperglycaemia. Treatment of the liver disorder was stated to be far more successful than insulin therapy and diabetic regime in such cases.

Hepatomegaly may occur in diabetes. It is generally considered to be due to gross fatty infiltration (LICHTMAN). According to MARBLE *et al.*, such enlargement of the liver is most common in poorly controlled diabetics. They found that with good control of the diabetic condition, a decrease in the size of the liver occurred in the majority of cases. STRÖM and I have had the same experience (LARSSON & STRÖM). A special form of hepatomegaly is that occurring in diabetic children, the so-called Mauriac syndrome, *i. e.*, diabetes, hepatomegaly, dwarfism, infantilism and obesity (see review by WINDORFER).

Although the fatty infiltration of uncontrolled diabetes may develop into fibrosis of the liver, manifest *cirrhosis of the liver* is not particularly common in this disease. However, BELL has stated, on the basis of an autopsy series, that diabetes is about five times as frequent in cirrhotic males as in the general autopsy male population. He found no convincing increase of diabetes in cirrhotic females. The extensive pancreatic lesions described in cirrhosis of the liver (LANDO; POGGENPOHL) are a conceivable explanation of the higher incidence of diabetes in this disease.

Cholelithiasis and *cholecystitis* tend to be more common among diabetics than in non-diabetic populations (LICHTMAN; MARBLE; WARREN & LECOMPTE). This may have different causes. One is a possible disorder of cholesterol metabolism in diabetes. The coincidence of the two diseases may also be due to a common familial disposition (CHIRAY *et al.*). The frequent occurrence of diseases of the gallbladder or bile ducts in pancreatitis is well known. Thus, in LAGERLÖF's series of 27 certain cases of pancreatic disease, 21 had signs of biliary tract disease. In the 5 cases of diabetes in this series, the bile ducts were, however, normal; consequently, in these 5 cases, the pancreatic disease had probably not been caused by gallbladder disease.

The occurrence of hyperglycaemia and glycosuria in cholecystitis has, however, been interpreted as a sign of secondary pancreatitis with damage to the endocrine tissue (BARBER; LICHTY & WOODS; RABINOWITCH & BAZIN). KATSCH found active cholecystitis or a history of this disease in 24 per cent

of a series of 677 diabetics, and also stated this to lend support to the view that diabetes may be secondary to cholecystopathy.

Even if symptoms of insufficiency of endocrine pancreatic function may appear in pancreatitis associated with biliary tract disease, the reverse has also been observed. Thus, MALLET-GUY has described two cases of *biliary tract disease, pancreatitis* and *hyperinsulinism.* One patient died, and autopsy showed hypertrophy of the pancreatic islets. The other patient recovered, but had a tendency to hypoglycaemia during the acute stage. LERICHE & SCHNEIDER reported a similar case of cholecystitis and hyperinsulinism, in which partial pancreatectomy was performed. Histologic examination of the portion removed showed signs of hyperplasia of the insular apparatus, but a normal exocrine parenchyma.

Improvement in diabetes after cholecystectomy has been reported by HARVIER & CAROLI. Their patient was a 50-year-old man, in whom the onset of diabetes (polyuria >3 liters, glycosuria 40—50 g/24 hrs) and cholelithiasis was simultaneous. His diabetic condition was controlled by 40 units of insulin a day; one month later, cholecystectomy was performed. Concurrent liver biopsy showed signs of subacute hepatitis and incipient cirrhosis. After operation, the diabetic symptoms regressed rapidly. Insulin could be discontinued, and the patient subsequently remained aglycosuric. A similar case was reported by RATHERY & FROMENT. Cholecystectomy for gallstones was performed in a 54-year-old woman with a history of diabetes for the past five years; she had constant glycosuria but was not taking insulin. Postoperatively, the glycosuria disappeared, in spite of increased carbohydrate intake. In both these cases, improvement in the diabetic condition was interpreted by the respective authors as an indication that the biliary tract disease had led to liver damage, which accentuated the disorder of carbohydrate metabolism.

Cases of precisely the reversed type have, however, been described, *i. e., improvement in diabetes with the onset of liver damage.* Thus, CAROLI *et al.* reported three cases of their own and collected an additional 10 reports from the literature, in which cirrhosis of the liver had led to disappearance of all diabetic symptoms, the duration of diabetes having been long in most of the patients. It does not seem possible to ascribe the improvement to a decreased food intake since—at any rate in the patients of CAROLI *et al.*—the general condition remained good, and a high-carbohydrate diet was given, in view of the liver damage.

The somewhat likely explanation that the liver damage would have resulted in inability of the liver to store glycogen and thus to maintain a high blood sugar level was rejected by CAROLI *et al.*, in view of the autopsy

findings in one of their patients, who died some years later of pneumococcal peritonitis. Examination of the pancreas then showed "une hyperplasie très nette des éléments insulaires de Langerhans", in some parts of the organ "une véritable néoformation d'ilots", with some islets lying outside the acinar parenchyma, in the interstitial adipose tissue. The exocrine parenchyma had a normal appearance. The authors stated that they were unable to explain their observations, although they suggested hormonal mechanisms dependent on the liver cell damage as a possible cause of the islet hyperplasia.

Unterhuber described a similar case, in which carcinoma of the liver developed in a 50-year-old man with insulin-treated diabetes of six years' duration. All the diabetic symptoms disappeared, and insulin therapy could be discontinued.

In this connexion, there is reason to review in more detail the investigations on the *effect of biliary fistula* mentioned in the introduction to this paper. In 1935, Leriche & Jung published certain experimental observations on the effect produced by biliary fistula on some endocrine organs, *i. e.*, the parathyroids, the adrenals and the thyroid gland. They found signs of hypertrophy with retention of secretion in both the thyroid and the parathyroids, whereas the changes were less marked in the adrenals. In the clinical part of this work, they reported the case of a woman who had earlier been operated on for goitre. When gallstones appeared, a biliary fistula was created, which functioned for two months. During this time, there was a recurrence of the goitre.

The effect of biliary fistula on the pancreas was reported in a separate communication in 1939. A biliary fistula was created in 17 dogs by introduction of a cannula into the gallbladder or the common bile duct. The bile flow to the intestine was interrupted by division of the common duct. The survival time of the animals ranged from 4 days to 4 months. In 7 out of 9 animals the fasting blood sugar was less than 90 mg/100 ml, which was stated to be the lower borderline for this value in normal dogs. Repeated intravenous glucose tolerance tests were made in one case. There was no marked rise in the blood sugar, and it had returned to the fasting level after 40 minutes. The glucose tolerance was distinctly increased in comparison to that in an untreated control. Histologic examination of the pancreas showed an increase in number and volume of the islets, and "une congestion vasculaire prononcée". No systematic quantitative examination of the pancreas was made. Slight fibrosis and a few necrotic foci were present in the exocrine parenchyma.

In connexion with these experimental observations, the following *clinical*

observation was made. Symptoms of diabetes appeared in a 31-year-old woman in August 1935; the diagnosis was made early in 1936, and dietary treatment was tried. Insulin therapy was started in early November, since dietary restrictions had resulted in no improvement. The insulin requirement was 80 to 100 units/24 hrs. The patient improved. In the beginning of 1937, insulin was discontinued and the diabetic symptoms reappeared. She was admitted to hospital in March. The blood sugar was then 525 mg/100 ml, and glycosuria 63.5 g/24 hrs. Glucose tolerance test: 260—247—225—300 (blood sugar). On April 17, a biliary fistula was created by cholecystostomy. A copious bile flow was obtained (about 300 ml/24 hrs). On the day after operation, 80 units of insulin were given, but none thereafter.

The patient was discharged from hospital in good condition; the fistula bag contained 200—300 ml of bile per 24 hrs. Despite no insulin, she had no diabetic symptoms, no thirst or increased hunger and felt "totalement transformée". A glucose tolerance test was made in July 1937: 184—236—168—188. There were only traces of sugar in the urine. In October, the blood sugar was 170 mg/100 ml. In April 1938 (12 months after creation of the fistula) the drain was removed and the fistula healed. Three months later the diabetic symptoms reappeared with the same severity as before; the urinary output was 2—3 liters/24 hrs with heavy glycosuria. It was necessary to give 90 units of insulin per day. In January 1939, the condition was unchanged; blood sugar 276 mg/100 ml, glycosuria 30 g and urinary output 2 liters/24 hrs.

The observations made by LERICHE & JUNG were confirmed by VERNE in 1940. He also used the dog as the experimental animal. The total size of the islet tissue was not calculated at histologic examination, but a numerical evaluation was made of the ratio of islets to acini by drawing on paper, in selected visual fields, cutting out and weighing islets and acinar tissue separately. When the biliary fistula operation was performed, a specimen was taken for biopsy. The ratio of islets to acini determined in this way was then 28 : 1,000; 17 days later, the corresponding ratio was 92 : 1,000. This was interpreted by the author as an enlargement in the islet tissue of about 3 times. The increase was more in the form of an increase in number ("polynésie") than of an increase in size ("macronésie"). The author discussed the results against the background of earlier experience, according to which starvation leads to an increase in islet tissue size. He nevertheless regarded it as established that, in his biliary fistula experiments, the increase in islet tissue was of a different type, both qualitatively and quantitatively, to that produced by starvation alone.

In a later publication, VERNE (1946) reported similar observations at autopsy of a 13-month-old child, who had a tumour which compressed the common duct. A biliary fistula was created through the gallbladder, and the child survived for a further 23 days. In this case, there was a considerable increase in the number of islets in both the head and the tail of the pancreas. In some parts, the islet tissue comprised about one-fourth of the pancreatic tissue. There was a marked predominance of beta cells in the islets. VERNE stated that he found numerous indications of acino-insular transition both in this child and in the biliary fistula dogs.

The occurrence of spontaneous *hypoglycaemia of hepatic origin* has been pointed out by several authors. Thus, JOSEPHS described 10 cases of hypoglycaemia in children with toxic liver damage; in two cases of phosphorus poisoning there was a fatal outcome owing to excessive fatty infiltration of the liver. COLLER & JACKSON reported three cases with severe disease of the gallbladder and bile ducts, secondary liver damage and typical attacks of hypoglycaemia, which disappeared after surgical treatment of the biliary tract disease. They emphasized that the glucose tolerance test is a valuable diagnostic tool in such cases, since it shows a low fasting value and a high curve of "diabetic" type. The same type of hepatic glucose tolerance curve has been described by SOSKIN & LEVINE and by CONN & SELTZER who, in a recent review, gave a complete aetiologic classification of spontaneous hypoglycaemia. BREIDAHL *et al.* stated, on the contrary, on the basis of their experience in 91 cases of hyperinsulinism, that the glucose tolerance test is of no diagnostic value.

MELLINKOFF & TUMULTY reported 20 cases of hepatogenic hypoglycaemia. The liver diseases responsible were virus hepatitis in 5 cases, Laennec cirrhosis in 5, congestive heart failure with congestion of the liver in 5, biliary cirrhosis in 2, fatty infiltration in 2, and primary hepatoma of the liver in 1 case.

Summary

1. In most cases of uncomplicated diabetes, there are no signs of liver dysfunction, and the liver plays no aetiologic role in the diabetic condition.

2. In gross lesions of the liver and bile ducts (hepatitis, cirrhosis, gallbladder disease), hyperglycaemia and glycosuria are relatively common, although the reverse, *i. e.*, spontaneous hypoglycaemia in hepatic disease, is also a known clinical feature.

3. Considerable improvement in pre-existing diabetes has been described in several cases in connexion with the onset of cirrhosis of the liver. A

similar observation has been reported in a case of biliary fistula and diabetes. In experimental biliary fistula, an increase in the size of the islet tissue has been described.

THE ISLET TISSUE IN PANCREATIC FIBROSIS AND PANCREATIC DISEASE

Experimental Observations

Production of pancreatic atrophy by ligation of the excretory ducts has been extensively used in experimental research. In earlier years, much of this work was done to test the so-called insular hypothesis, which is today mainly of historical interest (see review by ALLEN 1913). Most authors agree that the islets survive the exocrine parenchyma. Definite proof that this is so was given by BANTING & BEST, when isolating the first insulin preparation from a pancreas in which the duct was ligated. Opinions are, however, at variance with respect to the size of the islet tissue after ligation of the pancreatic duct.

An increase in the size of the islet tissue has been described by several writers. Thus, LAGUESSE & GONTIER DE LA ROCHE (1902) reported that when the excretory pancreatic ducts were ligated in guinea-pigs, the whole gland assumed an embryonic nature; the exocrine tissue atrophied, but the islets persisted, increased in size, and became more numerous by new formation from the epithelium of the ducts. On the basis of a theory that, after ligation of the duct, the acini become converted into islets, MANSFELD several years later studied the fasting blood sugar in dogs, in which half the pancreas had been ligated. As compared to normal dogs, the blood sugar was low, especially after several days' starvation, and histologic examination was stated to show a greater number of islets than in normal animals.

DE TAKÁTS & CUTHBERT shared MANSFELD's view that ligation of the pancreas results in increased sugar tolerance, owing to an increase in the islet tissue in the atrophic part of the organ. No improvement in the diabetic condition was, however, obtained in two diabetic children in whom such an operation had been performed. Similar mass ligation of the pancreas was done by KOSTER *et al.* in three patients with diabetes. In these cases as well, no change took place in the diabetic condition.

HERXHEIMER, who divided the pancreatic ducts in hens, also stated that he found signs of new formation of insular tissue from the atrophic

exocrine parenchyma. The effect of such hypertrophy of the islet tissue was particularly evident in an animal that died of hypoglycaemia one month after operation, the blood sugar being 19 mg/100 ml. Greatly enlarged islets ("geradezu ungeheure Zellinseln") were present in the pancreas of this animal, and determination of the insulin content of the organ showed a value five times greater than that in normal hens.

TERBRÜGGEN & HEINLEIN induced exocrine atrophy of the pancreas in rabbits by roentgen irradiation of the organ. No damage to the islets appeared; they were somewhat enlarged instead. The animals also exhibited a complete lack of glycogen in the liver and muscles, and an almost complete absence of adipose tissue throughout.

In their comprehensive articles, both BARGMANN and KRAUS seem to share the view that enlargement of the exocrine part of the pancreas through ligation of the excretory ducts leads to new formation of islets and to an increase in islet size.

ALLEN's opinion regarding the fate of the islets after duct ligation is the opposite of that of the authors cited in the aforegoing. In his experience, this procedure in the dog leads to dense sclerosis, which produces successive destruction of the islets by strangulation. He also emphasized that many errors have earlier been made in the microscopic study, owing to the difficulty of distinguishing in the atrophic organ between islets and involuting acinar tissue.

BEST (1934) stated that he did not find it possible to reproduce MANSFELD's experiments and that, in his experience, there are no signs of a total increase in the islet tissue of the dog after duct ligation. He found that some such dogs even became mildly diabetic three to four months after operation. Moreover, the insulin content of the pancreas is reduced after duct ligation, a fact that has also been stressed by HAIST.

The cause of these divergences in opinion is presumably to be sought partly in species differences. Another possible explanation is that, after ligation of the duct, both degenerative and regenerative processes may occur concurrently in the pancreas. The situation in the rabbit has been described as follows by BENSLEY.

"Towards the end of the first month regenerative changes become prominent, resulting in the formation of new acini and new islets of Langerhans from the remains of the duct system. At the same time the original islets not involved in the primary effect, that is to say, the larger islets, begin to be involved in the advance of the sclerotic process. The connective tissue invades the tissue of the islets themselves and the cells show to some extent atrophic changes." In a later stage "...the islet tissue is being

progressively increased by the addition of new islets developed from the duct system with which they remain in continuity, and the original islets for the most part atrophy as a result of the invasion of the connective tissue". According to BENSLEY, after more than five months practically all islet tissue is newly formed, and signs are present of a continued, progressive increase in this tissue.

Clinical Observations

A simultaneous disturbance in both the endocrine and exocrine function of the pancreas is generally considered to be a clinical rarity (ÅGREN *et al.*). The secretin test is usually normal in diabetes (LAGERLÖF). In an early paper by SSOBOLEW (1902), the islet tissue was stated to be normal in 17 cases of fibrosis of the pancreas, in contrast to 15 cases of diabetes, in which it was atrophic. Von GLAHN & CHOBOT studied the pancreas in 100 cases of chronic passive congestion, and found exocrine atrophy but intact islet tissue. The exocrine tissue was less damaged close to the islets than in the remainder of the parenchyma. These findings were explained by the authors as a result of the special type of blood supply in the islet tissue.

In gross lesions of the pancreatic tissue, particularly in obstruction of the ducts due to carcinoma or pancreatic lithiasis, there may, however, be damage to the insular apparatus as well, with resulting diabetes. One such case has been described by BARRON, the cause being pancreatic calculi, and five by LAGERLÖF, the cause being lithiasis in two cases, cancer in two, and acute destructive pancreatitis in one case. According to ZIMMERMANN, diabetes occurs in 12 to 13 per cent of cases of carcinoma of the pancreas, and in about 25 per cent of cases of chronic pancreatitis, with or without demonstrable stones.

In contrast to these observations, hyperplasia of the islets has been described by TERBRÜGGEN in two cases of carcinoma of the pancreas, and by BRINCK & SPONHOLZ in a case of pancreatic lithiasis in which hypoglycaemic symptoms were also present.

Diabetes has not been reported in *congenital cystic fibrosis of the pancreas* and the islet tissue is, as a rule, undamaged (ANDERSEN; BERGSTRAND; KAIJSER & LUNDQUIST; MAY). However, a decrease in the number of islets has been stated to occur in occasional cases, as well as hyperplasia in a few cases (BENOIT). The low carbohydrate tolerance in oral glucose tolerance tests that is so often present in this disease is presumably due, in most cases, to a disturbance in absorption. In two cases of congenital

fibrosis of the pancreas, I found normal or slightly increased tolerance in intravenous glucose tolerance tests (LARSSON). SEIFERT studied the ratio of alpha to beta cells in 12 cases of cystic fibrosis of the pancreas, and found a higher incidence of alpha cells than normally. The author stated this embryonic feature to be compatible with the general immaturity of the pancreas in this disease.

Summary

1. Opinions differ as to the size of the islet tissue after ligation of the pancreatic duct. Some authors hold the view that an increase in the islet tissue occurs, whereas others state the reverse to apply. Species differences may exist. It is also probable that degenerative and regenerative processes take place concurrently in the same animal. As far as it has been possible to ascertain, no quantitative evaluation of the islet tissue in this condition has been published.

2. According to clinical experience, insufficiency of the insular apparatus is rare in pancreatic disease, and occurs only in the presence of gross lesions of the gland, with extensive destruction of the exocrine parenchyma. In congenital pancreatic fibrosis, the islet function has been found to be normal.

EFFECT OF ALLOXAN AFTER LIGATION OF PANCREATIC DUCT

The effect of ligation of the pancreatic duct on the action of alloxan in the rabbit was investigated by WALPOLE & INNES. They found that if alloxan was given on the day after ligation, the same diabetogenic effect was obtained as in normal controls. If, on the other hand, alloxan was not given until 23 to 58 days after operation, no diabetic symptoms appeared. When the dose was raised to 400 mg/kg of body weight, the result was a slight initial rise in blood sugar, but no permanent diabetes. The authors therefore concluded that animals without functioning exocrine parenchyma are resistant to the diabetogenic effect of alloxan.

LABARRE & HANQUINET later performed similar experiments on dogs. Alloxan was given four to five weeks after division of the pancreatic duct, the dose being 70 mg/kg of body weight. All the animals became diabetic, and these authors were thus unable to confirm the results of WALPOLE & INNES. GOLDNER & GOMORI induced partial fibrosis of the pancreas by ligation of the duct in three dogs, and subsequently administered alloxan. Diabetes appeared in every case, and the beta cell necrosis was as pronounced in the atrophic as in the normal pancreas tissue.

Adams ligated the pancreatic duct in four dogs. Alloxan was given 67, 42, 39 and 96 days, respectively, after operation. The dose was 100 mg/kg in two cases and 75 mg/kg in two. Alloxan was also given to two controls; 100 mg/kg to one and 75 mg/kg to the other. Massive glycosuria appeared in both controls, and the blood sugar was about 500 mg/100 ml. The blood sugar values of the operated animals were normal: 111, 116, 147 and 95 mg/100 ml, respectively, and no glycosuria was present. Microscopic examination of the pancreas showed complete fibrosis and atrophy of the exocrine parenchyma but normal islets in the operated animals, whereas typical alloxan changes were seen in the islets of the controls. Adams was thus able to confirm the observations of Walpole & Innes. Partial fibrosis of the pancreas in rats with ligated pancreatic ducts was found by Hultquist to have no effect on the diabetogenic action of alloxan.

Similar experiments were performed by Cappellato & Perissinotto. They showed that injection of alloxan into the pancreatic duct of normal rabbits produced diabetes, and that with this mode of adminstration the dose is 10 times less than that required in intravenous administration. When alloxan was given in the same way intracanalicularly to animals in which the duct had earlier been ligated, the results were as follows. Diabetes appeared in all three animals in which ligation had been done 8 days previously. Of three animals in which the duct had been ligated 30 days earlier, one became diabetic, whereas all three animals operated on 50 days before alloxan administration were resistant.

The reason why Labarre & Hanquinet, Goldner & Gomori and Hultquist did not find resistance to alloxan in their experiments, as did the other workers quoted, cannot be definitely established. In the cases of the first-mentioned authors, the cause may, however, have been that alloxan was given before complete atrophy of the organ had occurred, and in the two last-mentioned that only partial fibrosis was present.

Various hypotheses have been put forward to explain alloxan resistance after ligation of the pancreatic duct. Walpole & Innes have stated it to be conceivable that the normal alloxan effect occurs as a result of a combination between alloxan and some secretory product in the actively functioning exocrine parenchyma. In this connexion, it may be recalled that the effect of alloxan has been shown to be greater in animals that have been starved on the day before administration than in those fed normally (Kass & Waisbren; de Moor). Adams has suggested that alloxan resistance may be due to the decreased blood flow in the fibrotic pancreas. It has, in fact, been shown by the investigations of Bailey *et al.* and by Gomori & Goldner, among others, that alloxan has no diabetogenic

effect if it is given during temporary occlusion of the arteries of the pancreas.

CAPPELLATO & PERISSINOTTO have suggested, on the other hand, that the absence of diabetogenic effect might be associated with the foetal nature of the islet tissue in the duct-ligated gland, since the alloxan effect is stated to be proportional to the degree of development of the insulin-forming tissue (*cf.* DE MOOR; HUGHES & HUGHES). These authors also mentioned the possibility of inactivation of alloxan in the abundant newly-formed connective tissue, supposed to be rich in sulphydryl groups (*cf.* LAZAROW).

DE MOOR, in accepting the conception that ligation of the pancreatic ducts leads to hyperplasia of the islet tissue, submitted that alloxan resistance might be due to this factor.

As pointed out in the introduction to this paper, FERNER put forward an essentially different explanation of alloxan resistance following ligation of the duct. He based his view on the known fact that absence of the external pancreatic secretion, as in depancreatized dogs, leads to an accumulation of excess fat in the liver, owing to lack of lipotropic factors, that are normally made available for absorption from dietary protein through action of the pancreatic juice (*cf.* BEST 1949). According to FERNER, this fatty infiltration of the liver results in inability of its parenchyma to produce hyperglycaemic blood sugar levels, even in the complete absence of insulin. He contended that alloxan given to animals with the pancreatic duct ligated results in beta cell damage of the same order of magnitude as in normal animals, but that the resulting diabetic condition is masked by the liver lesion. The condition is thus similar to the absence of diabetic symptoms after combined pancreatectomy and hepatectomy.

Mention can also be made in the present connexion of ALLEN's experiments, performed many years before the diabetogenic effect of alloxan was known. He found, in numerous experiments, that removal of seven-eighths to nine-tenths of the pancreas consistently led to diabetes when the ducts were left patent. On the other hand, diabetes did not develop after similar subtotal pancreatectomy when the external secretion of the pancreatic remains was blocked by ligation of the duct, at the same time that pancreatectomy was performed. ALLEN did not believe this to be due to presence or absence of pancreatic juice in the intestine, but rather to "some relation between the external and internal secretion in the gland itself".

Although he naturally regarded the islets as "the specialized tissue pre-eminently subserving the internal carbohydrate function of the pancreas", he stated it to be conceivable that the cells which, under normal con-

ditions, form the external secretion, have a possibility—once external function ceases—to "direct their energies to the production of internal secretion".

BENSLEY—in a comment on these investigations by ALLEN—shared his view in so far as the intervention favours mitosis in the pancreas cells and leads to increased formation of new islet tissue, which produces a gradual rise in carbohydrate tolerance. He nevertheless also emphasized that the decreased efficiency of carbohydrate digestion resulting from cessation of external pancreatic secretion implies a smaller load on the islet cells than normally.

Summary

According to several workers, alloxan has no diabetogenic effect if given to animals with fibrosis of the pancreas due to ligation of the duct. Although several hypotheses have been put forward, no definite explanation of this fact has been given.

Subtotal pancreatectomy has been found to be less apt to lead to diabetes when external secretion of the pancreatic remains is blocked by ligation of the duct than when the ducts are patent.

FACTORS AFFECTING THE SIZE OF THE ISLET TISSUE

A comprehensive review of the literature on this subject has been given by several authors (BARGMANN; GOMORI; HAIST; TEJNING). As a rule, the factors which affect the size of the islet tissue belong to one of two categories: dietary or hormonal.

Dietary Factors

A thorough study of these factors has been made by TEJNING, in an experimental investigation on the effect of different diets on the quantitative morphology of the pancreatic islets of the rat. He found the highest values for islet volume (expressed in mm^3 per 100 g of body weight) with a high-carbohydrate diet, and the lowest with a high-fat diet. Intermediate values were obtained with a natural diet and with a high-protein diet. These results are in agreement with the investigations of BEST *et al.* on the insulin content of the pancreas, in which both starvation and a high-fat diet led to a considerable decrease in the insulin content of the organ. In

addition, Ashworth *et al.* and Haist *et al.* have shown that long periods of undernutrition prevent growth of the islets in young rats, whereas this does not apply to complete starvation of short duration.

Opinions are, however, divergent as to the effect of starvation, some authors claiming that the islet tissue increases in size, and others the contrary. The reason may be a lack of sufficiently accurate methods, since in most cases the effect of starvation was evaluated qualitatively only. An increase in islet tissue may be merely apparent, and be due to the fact that, in starvation, the acinar tissue decreases more than the islet tissue, so that the islets seem to be more numerous than normally, although no increase has actually occurred (Bargmann; Jackson). This source of error applies in particular in determinations of the ratio of insular to acinar tissue.

Glucose Administration

The effect of glucose administration on the size of the islet tissue has been studied by several workers in different species and with various modes of administration. In most of these investigations, glucose could be shown to lead to hyperplasia of the islets. Thus, Corpaci gave 20 ml of 25 per cent glucose solution daily by the intraperitoneal route to four rabbits for two months, and found a marked increase in volume of the individual islets. Four animals given daily insulin injections during the same period exhibited a marked decrease in islet size. Similar results were reported by Miyamoto, who gave glucose orally to rabbits over a long period, and noted hyperplasia of the islet tissue parallel to increased glucose tolerance.

Houssay *et al.* found, in rats, that intraperitoneal injection of glucose led to hyperplasia and hypertrophy of the islets, and also prevented appearance of diabetes in subtotal pancreatectomy. Wissler *et al.* noted islet hyperplasia in six rats that were force-fed with a high-carbohydrate diet for 56 days. Haist *et al.* as well found a large increase in the islet volume in rats even after 7 to 10 days' glucose administration. They also demonstrated that the glucose effect was confined to the islet tissue, the total pancreas weight being the same in the treated animals as in the controls. According to Haist, the effect of glucose depends more on the total quantity administered than on the rise in blood sugar, which is usually only transient.

The effect of glucose in the guinea-pig has been studied by Woerner, among others. He found that continuous intravenous administration for a

relatively short period led to an extensive increase in the islet tissue, with new formation of beta cells from existing beta cells, but also from the duct and acinar cells. The effect of a single injection of glucose was studied by GOMORI *et al.* and by PETERSON. They observed varying degrees of degranulation of the beta cells. Degranulation was also a common finding in the aforementioned experiments with repeated or continuous glucose injections.

BARRON & STATE gave continuous intravenous injections of glucose to dogs for 4 to 9 days, and found total degranulation of the beta cells, and in one animal numerous diffuse haemorrhages and necroses of the islets as well.

DOHAN & LUKENS' investigation of the glucose effect in the cat is often cited. Glucose was injected intraperitoneally into 35 cats, and partial pancreatectomy was performed in 8 of them. Hydropic degeneration of the beta cells resulted in 10 cases; 3 of these animals exhibited "persistent diabetes". Partial pancreatectomy had been performed in 2 of these 3 animals. One of these animals died of diabetic acidosis, one died of an unknown cause 8 days after cessation of glucose administration, and glycosuria disappeared terminally in the third. These experiments indicate that excessive glucose administration *may* lead to exhaustion of islet function. It can, however, be recalled that no changes in the islet tissue were visible in the majority of animals in DOHAN & LUKENS' series.

Hormonal Factors

Insulin inhibits growth of the islets (CORPACI; HAIST *et al.*) and may even, under certain conditions, produce islet atrophy, which leads to diabetes (MIRSKY *et al.*).

The effect of *anterior pituitary hormones* on the size of the islet tissue has been investigated by several workers. Thus, RICHARDSON & YOUNG found an increase in the islet tissue in rats after administration of a crude extract of the anterior pituitary. Similar results were obtained by GÜTHERT and by CONN & LOUIS, who pointed out that the diabetogenic effect of anterior pituitary extracts may be due to *excessive* stimulation of the insulotropic principle of such extracts. Growth hormone also stimulates growth of the islet tissue (KINASH *et al.*), whereas hypophysectomy prevents growth of the islets (BRYANS *et al.*).

In contrast to the morphologic effect, it has been shown by BEST *et al.* that the insulin content of the pancreas is lowered by administration of anterior pituitary extracts, although it could not be determined whether

this decrease was due to an increased liberation or to a decreased production of insulin. CAMPBELL *et al.* found that if the hyperglycaemia produced by the pituitary extract was abolished with insulin, there was no reduction in the insulin content.

Thyroid administration results in an increase in the size of the islet tissue (HAIST *et al.*).

The islet tissue is said to be increased in *pregnancy* (BARGMANN). The hyperplasia of the islets of *infants of diabetic mothers* could to some extent be reproduced experimentally in the offspring of diabetic rats by HULTQUIST. The mechanism of this hyperplasia is unknown, but it has been presumed to be a result of hormonal influences from the diabetic mother, possibly the growth hormone (HULTQUIST & ENGFELDT).

The hyperplasia of the islets observed in *erythroblastosis* may also be due to hormonal factors, since generalized endocrine dysfunction has been described in this condition (RANSTRÖM). An excessive increase in number and size of the islets was reported by DONOHUE in a case of "dysendocrinism".

Marked atrophy of the islets is a typical feature of *alloxan diabetes* (DUFF; LUKENS).

The literature on clinical hyperinsulinism and islet-cell tumours is beyond the scope of the present survey.

Summary

An increase in the islet tissue has been observed to result from high-carbohydrate diets, from glucose administration, and from administration of anterior pituitary extracts or hormones.

Inhibition of growth or a decrease in islet tissue has been found in connexion with undernutrition, insulin administration, hypophysectomy, and alloxan diabetes.

The mechanism responsible for islet hyperplasia is unknown, although it seems as if factors which stimulate the islet tissue are of such a nature that they lead to an increased requirement of endogenous insulin (HAIST). Given in excessive quantities, agents which stimulate the islets to growth and secretion may lead to exhaustion and degeneration of the islet cells, and to diabetes (HAIST).

CHAPTER 2

MATERIAL

The experimental animals consisted of 144 rabbits (77 males and 67 females); 139 of them were white domestic and 5 were chinchillas. On practical grounds, it was unfeasible to obtain a uniform strain by breeding pedigree animals in the laboratory. Consequently, the material was not homogeneous from the genetic viewpoint. The rabbits were, however, bought as far as possible from the same commercial breeder. Moreover, in order to avoid any great individual variations, all available animals from the same litter were bought on each occasion. Only adult rabbits in good general condition were accepted. With few exceptions, their weight ranged from 2.0 to 3.5 kg at the beginning of the experiments, and their age from 5 to 7 months.

GROUPING

The animals were divided into three experimental groups and one control group as follows (*cf.* Table 1).

Group I (25 animals): creation of a biliary fistula.
Group II (29 animals): ligation of the common bile duct.
Group III (34 animals): ligation of the pancreatic duct.
Group IV (56 animals): controls.

Supplementary material.—In addition to these 144 animals, 5 untreated rabbits were investigated with respect to their glucose tolerance. The results are included in the "normal glucose tolerance" group (p. 42). No other investigations were made on these five animals.

Thus, in one respect, there was an essential similarity between groups I and II, since in both of them the normal bile flow to the intestine was completely occluded. These two groups are therefore discussed under the common heading of "bile flow experiments".

Group I was divided into two sub-groups: A and B, and groups II and III into three sub-groups: A, B and C. In the *A groups,* the effect of the relevant operation was studied in non-diabetic animals. In the *B groups,*

TABLE 1

The material

	No. of animals Category 1	Category 2	Total
I. *Biliary fistula*			
A. Non-diabetic animals	9	10	19
B. Alloxan-diabetic animals	5	1	6
II. *Ligation of common bile duct*			
A. Non-diabetic animals	11	11	22
B. Alloxan-diabetic animals	3	2	5
C. Alloxan after bile duct ligation	—	2	2
III. *Ligation of pancreatic duct*			
A. Non-diabetic animals	10	4	14
B. Alloxan-diabetic animals	5	—	5
C. a) Alloxan 68 days after ligation of pancreatic duct	9	—	9
b) Alloxan 11 days after ligation of pancreatic duct	—	6	6
Total	52	36	88
IV. *Control groups*			
A. Untreated animals	4	—	4
B. Pair-fed controls to group I A	9	—	9
C. Pair-fed controls to group II A	4	—	4
D. Control operation (ligation of left ureter)	4	—	4
E. Underfed animals	4	—	4
F. Glucose administration			
a) Glucose alone	6	—	6
b) Alloxan after glucose	—	6	6
G. Alloxan-diabetic controls			
a) Alloxan dose 200 mg/kg BW	4	7	11
b) Alloxan dose 100 mg/kg BW × 2	5	3	8
Total	40	16	56
Whole material	92	52	144

the same effect was studied in animals in which diabetes had been induced preoperatively by administration of alloxan. In the *C groups,* the reverse procedure was applied, *i. e.,* alloxan was given at a certain interval after operation, in order to ascertain whether its diabetogenic effect was influenced by the intervention. Group III C was further sub-divided into two groups. In one of them (*group III C a*), the effect of alloxan was studied at a relatively long interval (mean 68.4 days) after ligation of the pan-

creatic duct. In the other (*group III C b*), the effect of alloxan was tested already 11 days after operation.

The control material (*group IV*) comprised 7 sub-groups. *Group IV A* consisted of 4 entirely healthy and untreated rabbits, who were given the full standard diet of the laboratory for several weeks.

Groups IV B and IV C require a special comment. In the bile flow experiments made in groups I and II, it was obviously impossible as a rule to prevent the general condition being affected. This was most evident in the form of decreased appetite and loss of weight. Operation thus led to a more or less prolonged period of undernutrition. Consequently, the animals were not comparable with untreated animals given the full standard diet. *Group IV B* therefore consisted of 9 rabbits, each of the same sex and from the same litter as an experimental animal in group I A. The two groups thus comprised 9 pairs of comparable animals. During the whole experimental period, the animal in group IV B was given the same quantity of food as that eaten by the corresponding operated animal in group I A. The undernutrition factor was thus the same in both groups.

My original intention was for *group IV C* to comprise a corresponding pair-fed control group to group II A. However, owing to the relatively high mortality associated with these bile duct obstruction experiments, I was unable to obtain more than 4 complete such pairs of animals. In order to provide a larger number of controls to group II A, I therefore formed *group IV E*. It consisted of 4 healthy and untreated rabbits who, during a certain period, were underfed to about the same extent as the corresponding experimental animals in group II A.

In contrast to interventions on the biliary tract, ligation of the pancreatic duct has been found to have no influence on the general condition, appetite or body weight of the animal, despite interruption of external pancreatic secretion. Consequently, I considered it superfluous to have a special group of pair-fed controls to group III. However, in order to ascertain whether the intervention had any intrinsic effect on the morphology of the pancreas, ligation and division of the left ureter was performed on 4 animals as a control operation (*group IV D*). Despite the appearance of unilateral hydronephrosis, this operation had no effect on the general condition of the animals. Group IV D was therefore regarded as a comparable control group to group III A, in addition to group IV A.

Enlargement of the pancreatic islet tissue could be demonstrated in groups I A and II A. I therefore considered it of interest to compare this enlargement and that stated by some workers to occur in connexion with glucose administration (see p. 30). This was investigated in *group*

IV F a. The effect of alloxan on animals given glucose was studied in *group IV F b.*

Finally, *group IV G* consisted of 19 alloxan-diabetic rabbits, controls to the animals in groups I, II and III given alloxan. In the first experiments, the alloxan dose was 200 mg/kg of body weight. This dose was found to cause a considerable loss of animals owing to toxic effects. The dose was therefore changed to 100 mg/kg of body weight, given on two consecutive days; the total dose was thus unaltered. Both types of dosage were used in experimental groups I, II and III. Consequently, control group IV G contained two sub-groups: *group IV G a*, consisting of 11 rabbits given 200 mg/kg in a single dose, and *group IV G b*, comprising 8 rabbits given 100 mg/kg two days in succession.

CATEGORIES

The animals in the various groups were assigned either to category 1 or 2. *Category 1* consisted of the 92 animals in which a more or less complete quantitative micromorphologic examination of the pancreas was made. *Category 2* comprised the remaining 52 animals in which no such examination was performed. In some cases this was because it was considered to be of no direct interest, *e. g.* in group III C b or IV F b. In others, the animal had died, and post mortem changes prevented any detailed microscopic study. In still other cases, the animal had died before a sufficiently long period had elapsed after operation or other form of treatment. This applied, for example, to animals in group II A or IV G a.

SEX AND BREED DISTRIBUTION

Sex

Males and females were present in all the groups with the exception of the two small groups II B and II C, which consisted of males only. There was not, however, an even sex distribution in the different groups. Since no systematic difference existed between the sexes with respect to the results of the investigation, this matter is presumably of no importance. Nor has the sex factor been taken into consideration in an account of the results.

Breed

As already stated, 5 of the rabbits (R. 119, 124, 125, 126 and 127) did not belong to the white domestic breed, but were chinchillas. The reason was that the breeder was temporarily unable to supply adult rabbits of the white type. Only three of the chinchillas (R. 125, 126 and 127) belonged to category 1. They were all from the same litter; 2 of them were included in group II A, and the third in the corresponding control group IV C. The results of the investigation did not indicate that these animals exhibited any general tendency to deviate systematically from the other animals in these two groups. Consequently, I found no reason to exclude them.

To sum up, the following statements can be made with respect to the material as a whole. It is true that the number of animals in each of the main groups and sub-groups was not particularly large, but a limitation was necessary in view of the fairly time-consuming micromorphologic analyses. On the other hand, the animal material was, on the whole, uniform and the experimental procedure was the same in the relevant groups. Consequently, I considered it possible to make a statistical comparison between them, despite the relatively small number in each.

CHAPTER 3

METHODS

GENERAL

The rabbits were kept in individual wire-mesh cages with an approximate volume of 100 dm^3. Simple metabolic cages were used, which permitted complete and continuous collection of the urine for analysis. The bottom of these cages consisted of a coarse metal grating, under which a funnel-shaped drain, provided with closely meshed wire netting for separation of the excrement and food leavings, collected the urine in narrow-necked glass bottles.

During the whole experimental period, the general care of the animals, cleaning of the cages and feeding were entrusted to the same assistant.

FEEDING

All the animals were given the same diet throughout the experimental period. It consisted of natural foodstuffs, the quantity per day being constant, *i. e.*, 100—130 g of clover hay, 75 g of whole oats, 100—130 g of swedes and 100—130 g of carrots. The animals were fed once a day. At the beginning of the investigation, the size of the portions was fixed by weighing. Thereafter, the quantity of food given to each animal per day was estimated approximately on this basis. Fresh drinking water was given each day *ad libitum*.

DETERMINATION OF BODY SIZE

The size of the rabbits was determined by weighing, the body weight (BW) being given with an accuracy of $\pm$ 0.01 kg. On the basis of the weight, the body surface (BS) was calculated with the formula given by Sм[illegible]rs:

$$BS = 56.33 \times BW^{0.436}$$

The body surface was determined with an accuracy of $\pm$ 0.1 dm^2.

URINALYSES

Volume.—The volume of urine was measured and expressed as ml/24 hrs.

Glucose.—Glycosuria was evaluated by Benedict's qualitative and quantitative tests and recorded as grams of glucose excreted per 24 hrs.

Ketone bodies.—The presence of ketone bodies in the urine was determined with a modification of the sodium nitroprusside test (Rothera's test). A reagent powder of the following composition was used:

Sodium nitroprusside 0.2 g
Dry sodium carbonate 44.0 g
Ammonium sulphate 55.0 g

Protein.—The urine was tested for the presence of protein with sulphosalicylic acid, using reagent granules (Cargille).

Bilirubin.—Some urine samples of animals in groups I and II were tested for the presence of bilirubin, using Fouchet's reagent, as described by HARRISON.

Diastase.—WOHLGEMUTH's method was used for testing some urine samples of animals in group III for the presence of diastase.

BLOOD SUGAR DETERMINATIONS

The blood sugar was determined with HAGEDORN-JENSEN's method in all the glucose tolerance tests. In addition, a few determinations for general information were made with FOLIN's micro-method, which is the routine method at our clinic. In every case, blood was taken from a marginal vein of the ear, vasodilatation having been produced by means of xylene.

Two blood samples were taken on each occasion, and the mean value of this double determination was taken as the blood sugar value.

GLUCOSE TOLERANCE TEST

Technique

The glucose tolerance was determined by means of an intravenous tolerance test. No food was given for 24 hours before the test. It was always made at the same time of day (between 10 and 11 a. m.) and usually on two animals at once. The dose was 0.4 g/kg of body weight of glucose in 25 per cent solution. The injection was completed in at most one minute. Samples

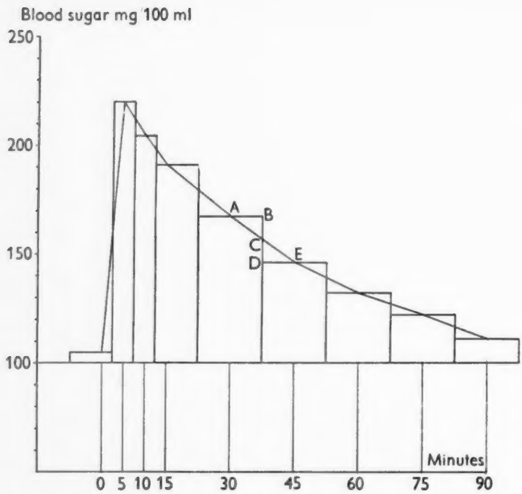

Fig. 1. Calculation of glucose tolerance area. Conversion of glucose tolerance curve into a histogram.

were taken for blood sugar determinations immediately before injection of glucose (0 min) and 5, 10, 15, 30, 45, 60, 75 and 90 minutes after completion of the injection. The glucose was always injected into a marginal vein of the left ear, whereas the samples were taken from a vein of the right ear.

Evaluation

The glucose tolerance test was evaluated by calculating the *glucose tolerance area*, expressed in *milligram-minutes* (mg-min). In place of the planimeter method of HIMSWORTH and ROSS & TONKS (*cf.* p. 62), which is fairly laborious if a large number of curves are to be measured, the following method was used (Fig. 1).

Instead of allowing the surface to be delimited by the blood sugar curve with its even course, the tolerance area is converted into a type of histogram, consisting of 9 rectangles. The level corresponding to the blood sugar value 100 mg/100 ml is taken as the baseline. The height of each rectangle represents one blood sugar value. Its breadth is the sum of half the time distance (in min) between this value and that directly preceding it and between the former value and that directly following it. Since the time interval between the blood sugar values is not the same throughout, the breadth of the rectangles ranges from 5 to 15 minutes. The rectangles corresponding to the endpoints of the curve (0 and 90 min values), and for

which directly preceding and following values are thus lacking, are given a breadth of 10 and 15 minutes, respectively. The area of each rectangle is given in mg-min as the product of its breadth in min and its height in mg/100 ml, and the total tolerance area as the sum of the areas of the individual rectangles.

An example of the calculation of the glucose tolerance area with this method is shown in Table 2. The blood sugar value (*a*) decreased by 100

TABLE 2

Example of calculation of glucose tolerance area (*cf.* Fig. 1)

Minutes	a Blood sugar mg/100 ml	b a−100	c Time factor	d Tolerance area, mg-min = b×c
0	105	5	10	50
5	220	120	5	600
10	205	105	5	525
15	191	91	10	910
30	167	67	15	1,005
45	146	46	15	690
60	132	32	15	480
75	122	22	15	330
90	111	11	15	165

Total tolerance area = 4,755 mg-min

TABLE 3

Intravenous glucose tolerance curve of 55 normal animals. Blood sugar, mg/100 ml. Mean curve, 95 per cent limits of variation ($t_{60} = 2.000$) and 95 per cent fiducial limits of the mean (*cf.* Fig. 2)

Time (Minutes)	0	5	10	15	30	45	60	75	90	Tolerance area (mg-min)
$+2\,s$	126.8	269.3	246.3	229.4	206.4	185.5	166.5	154.1	137.0	7,834
$+2\,e(\bar{x})$	108.0	226.6	210.1	196.2	172.7	151.5	136.7	126.9	114.4	5,195
$\bar{x}$	105.1	219.9	204.5	191.0	167.4	146.2	132.0	122.2	110.8	4,784
$-2\,e(\bar{x})$	102.2	213.2	198.9	185.8	162.1	140.9	127.3	117.5	107.2	4,373
$-2\,s$	83.4	170.5	162.7	152.6	128.4	106.9	97.5	90.3	84.6	1,734
s	10.86	24.70	20.89	19.22	19.50	19.66	17.27	15.93	13.10	1,525
$e(\bar{x})$	1.46	3.33	2.82	2.59	2.63	2.65	2.33	2.35	1.78	206
n	55	55	55	55	55	55	55	46	54	55

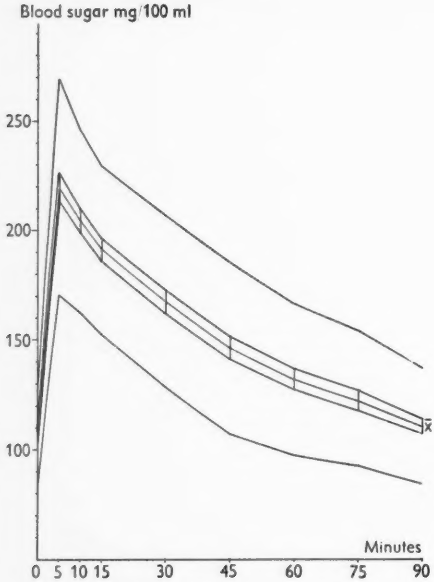

Fig. 2. Intravenous glucose tolerance test in 55 untreated animals: "the normal group". Mean curve ($\bar{x}$), 95 per cent fiducial limits of the mean, and 95 per cent limits of variation (± 2 s).

gives the height of the rectangle (b), since the blood sugar value 100 mg/100 ml is the baseline of the tolerance area. The time factor (c) comprises the breadth of the rectangle, and the area (d) is obtained as $b \times c$.

Using this method, the normal glucose tolerance was determined in 55 animals belonging to different groups, before they underwent any other experimental procedure. The results are recorded in Table 3 and Fig. 2. The mean value of the tolerance area is 4,784 $\pm$206 mg-min, but there is a considerable variation, so that the range, given as the 95 per cent limits of variation, amounts to 1,734 to 7,834. The evaluation of the glucose tolerance in the different experimental groups was made as a test of the possible deviation from these "normal values" (*cf.* Table 26).

The results of the glucose tolerance tests in the individual animals in the different groups are assembled in Tables I: 1 to I: 12 (Appendix).

ALLOXAN ADMINISTRATION

The preparation used was alloxan monohydrate (Eastman Organic Chemicals, no. 1722, mol. weight 160.09). The dose was 200 mg/kg of body weight, either in a single dose or in two doses of 100 mg/kg on two consecutive days. In some alloxan-resistant cases the dose was increased to

300 mg/kg of body weight. A freshly prepared 5 per cent aqueous solution was used, except with a dose of 300 mg/kg, when a 7.5 per cent aqueous solution was given. It was always given intravenously and at the same time of day (between 10 and 11 a. m.). The animals were starved for 18 to 20 hours before administration of alloxan, but were allowed to eat as usual 1 to 2 hours after the injection. In every case, 20 ml of a 30 per cent glucose solution were injected subcutaneously 6 to 8 hours after administration of alloxan.

The urine was analyzed daily with respect to volume, glycosuria, acetonuria and proteinuria, both for several days before administration of alloxan and continuously thereafter.

Insulin therapy was started as soon as diabetes was diagnosed. Regular and/or protamine insulin of the NPH type was used. It was given in sufficiently large doses to keep the urine free from acetone and the glycosuria about 10 g/24 hrs, if possible. No attempts were made to produce aglycosuria.

OPERATIVE TECHNIQUE

Anaesthesia. General

All operations were performed under strictly sterile conditions. The anaesthetics used were a short-acting intravenous barbiturate, sodium cyclohexenyl-allyl-thiobarbiturate (Kemithal, ICI), and ether. The former, of which the pharmacology has been described by Carrington & Raventós, was given in 5 per cent solution. Induction of anaesthesia was produced by an average dose of 50 mg/kg of body weight. When required, one or several additional 50-mg doses of kemithal were given intraperitoneally during operation. This type of anaesthesia was used mainly in the biliary fistula interventions in group I A. In the others, ether was generally given. In a few cases a combination of the two anaesthetics was used; anaesthesia was then inducted with ether and maintained with kemithal injected intraperitoneally.

The abdomen was opened by a midline incision. In the first experiments, a transverse incision was used, but it was soon found that a midline incision, which is made more rapidly and requires ligation of only a few blood vessels, gave equally good access to all the organs on which intervention was made. After operation, the abdominal wall was closed by a double layer of sutures, and the wound covered with absorbent cotton and collodium. The operative wound healed by first intention in every case.

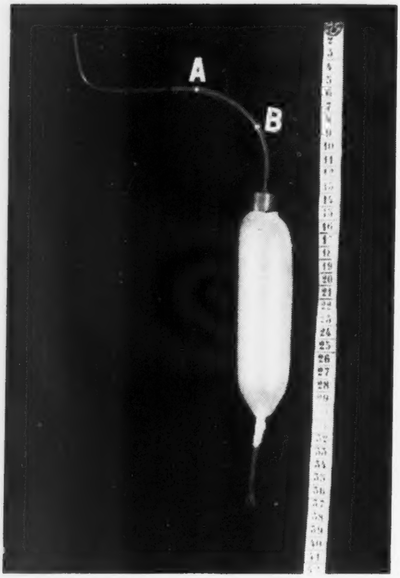

Fig. 3. Tubing system used in biliary fistula experiments, consisting of polyethylene catheter, rubbing tubing, thin-walled rubber bag and short tube for drawing off the bile. The system lay intra-abdominally up to point A, and subcutaneously from A to B.

Biliary Fistula

After the common bile duct had been ligated twice beside the duodenum and divided between the ligatures, the gallbladder was sought and a tobacco-bag suture about 5 mm in diameter passed around its fundus. Since the gallbladder of the rabbit is extremely thin-walled, it was necessary to use a special type of surgical suture with attached Deknatel "minimal-trauma" needle. The cannula of the drainage tube was introduced into the gallbladder through a 3 to 4 mm incision, and the suture tied tightly around it. In most cases there was no leakage of bile into the abdominal cavity, and a copious flow through the tubing started at once.

The tubing used in these experiments is shown in Figs. 3 and 4. Initially, a short glass cannula was used closest to the gallbladder, but polyethylene tubing was soon found to be more satisfactory. It had an inner diameter of 1.77 mm and an outer diameter of 2.80 mm. The flange at the end of the tube was produced by holding it close to a gas flame for a few seconds. The flange prevented the tube from slipping out of the gallbladder. The tubing was about 10 cm long, and was bent at an angle of approximately 90° about 3 cm from the opening in the gallbladder. While still in the abdominal cavity, it was connected to a rubber tube of about

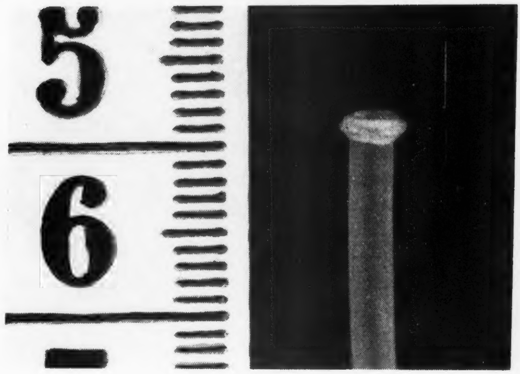

Fig. 4. Close-up of the receiving end of the polyethylene catheter. The circular flange at the tip prevents the catheter from slipping out of the gallbladder.

the same size. The latter was then drawn out of the abdominal cavity through an oblique incision in the left side of the abdominal wall. The rubber tube thus passed subcutaneously for a length of 3 to 5 cm. The part of the tube outside the animal's body was introduced into a thin-walled rubber bag. The tube was provided with a few lateral holes close to the hole in its tip.

The bag was kept in position by an abdominal girdle of strong cloth (Fig. 5). It was emptied at a fixed time each day, and the quantity of bile recorded in ml/24 hrs. To prevent infection of the bile, 2 ml of 0.1 per cent alkyl-dimethyl-benzyl ammonium chloride solution (Desivon, Astra) were injected into the bag daily.

Fig. 5. R. 216 (group I B) with alloxan diabetes and biliary fistula. Illustrates type of bandage used for bile bag.

Each day, the animals received subcutaneously, in a quantity corresponding to the 24-hr quantity of bile in the bag, a solution of the following composition:

Sodium chloride	5.51 g
Potassium chloride	0.37 g
Sodium bicarbonate	3.36 g
Distilled water Ad	1,000 ml

which corresponds in mEq/liter to:

Na^+	134	Cl^-	99
K^+	5	HCO_3^-	40
	139		139

The electrolyte analyses of the fistular bile were performed with the following methods. Sodium and potassium were determined with the Weichselbaum-Varney flame photometer, chloride with a modification of BRUN's method, bicarbonate with van Slyke's titrimetric method modified for electrometric titration, and glucose with Folin's micromethod*.

Ligation of Common Bile Duct

Two ligatures were applied to the common bile duct beside the duodenum, and it was divided between the ligatures.

Ligation of Pancreatic Duct

The pancreatic duct was easily identified at its opening into the duodenum, 10 to 15 cm from the pylorus on the ascending limb of the duodenal loop, at the level of the short peritoneal ligament running between the duodenum and the rectum. The duct was ligated twice beside the duodenum, and divided between the ligatures.

Ligation of Left Ureter

Although imbedded in considerable quantities of fat, no difficulty was encountered in identifying the ureter, close to the renal pelvis. It was ligated twice and divided between the ligatures.

* All these determinations were performed at the Central Laboratory of S:t Eriks Sjukhus.

AUTOPSY

The animals were stunned by a blow behind the ears; the vessels of the neck were then severed to produce exsanguination. They were always killed at the same time of day (between 4 and 7 p. m.). The body weight was determined immediately before death.

Autopsy was performed immediately after death. After the general observations had been made, *i. e.*, nutritional state, presence of jaundice, extent of visible adipose tissue, and any pathologic changes, the pancreas was dissected out. The caecum, appendix and colon were first freed by blunt dissection from their loose attachment to the mesoduodenum and pancreas. When the pancreas had thus been exposed, it was carefully dissected out. Particular care was taken to detach every part of it: the duodenal, the retroventricular and the splenic, as well as that part lying between these three, close to the main trunk of the portal vein, the left adrenal and the mesenteric root. The organ was freed as far as possible from surrounding adipose tissue.

The pancreas was then divided into two parts: one larger, duodenal part (part D) and a smaller, splenic part (part L): see Fig. 6. The portal

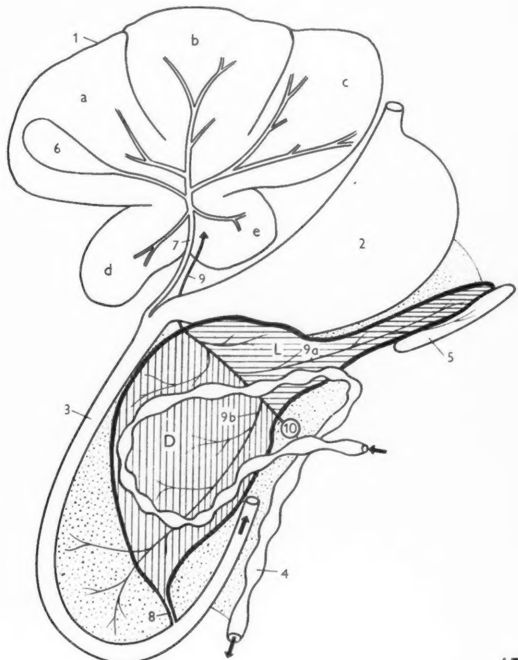

Fig. 6. Schematic drawing of the topographical anatomy of the rabbit pancreas (cf. Müller*).*

1. Liver
a. right central lobe
b. left central lobe
c. left lateral lobe
d. caudate lobe
e. papillary lobe
2. Stomach
3. Duodenum
4. Rectum
5. Spleen
6. Gallbladder
7. Common bile duct
8. Pancreatic duct
9. Portal vein
a. vena pancreatico-linealis
b. vena pancreatico-duodenalis
10. Mesenteric root.
D = duodenal part of pancreas.
L = splenic (lienal) part of pancreas.

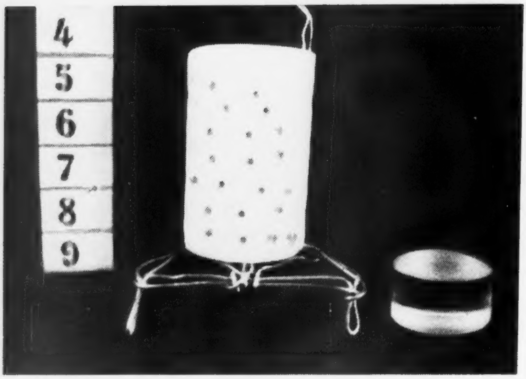

Fig. 7. Perforated china vessel used in fixation of the pancreas, and flat cylindric glass lid.

vein, which passes through the pancreas region towards the porta hepatis was taken as the approximate borderline. The two parts were then weighed separately with an accuracy of ± 0.01 g.

Specimens of tissue from other organs were removed for analysis as described in the following.

HISTOLOGIC AND HISTOCHEMICAL TECHNIQUE

Preparation of Pancreas

After weighing, the two parts of the pancreas were placed in Bouin's fluid for about 5 minutes. After this preliminary fixation, each part was placed separately in a cylindric, perforated china vessel with an inner diameter of 23 mm (Fig. 7). The elongated pieces of tissue were packed as tightly as possible, and the whole vessel was then lowered into a larger vessel, containing 168 ml of freshly prepared Bouin's fluid. The inner vessel was placed on a small metal stand, to permit the fixative to penetrate the organ from below as well. A cylindric glass lid, 21 mm in diameter, 11 mm high and weighing 10 g, prevented the pieces of pancreas from floating up.

After fixation for 24 to 36 hours, the pieces of tissue were imbedded in paraffin and cut in sections 5 μ thick. Serial sections were made of the whole organ. The sections were stained according to Gomori, with chrom-alum haematoxylin phloxine (except in one case: R. 14, group I B, see Chap. 6).

Other Organs

Thin slices of the liver and kidneys were fixed in absolute alcohol for glycogen staining with Best's carmine stain. Small pieces of tissue from the liver, kidneys and duodenum were fixed in cold acetone and then treated with GOMORI's method for demonstration of alkaline-phosphatase. Pieces of the liver and adrenal gland were fixed in 10 per cent formalin, after which frozen sections were stained in Sudan III according to Daddi, for determination of lipids. In many cases, specimens were also taken from the liver and kidneys and, if required, from other organs as well for staining with haematoxylin and eosin.

In evaluating the glycogen, alkaline phosphatase and lipid content of the different organs, the following approximate grading was used:

0: none
1: very low
2: low
3: low to moderate
4: moderate
5: fairly high
6: high
7: very high

In all histologic treatment of the material, generally accepted principles were used (GOMORI; LILLIE; PEARSE; ROMEIS).

QUANTITATIVE MICROMORPHOLOGIC ANALYSES

Size of Total Pancreas Parenchyma

Calculation of the size of the pancreas parenchyma was performed in 7 groups: I A, II A, IV A, IV B, IV C, IV D and IV E. The following method was used.

Sections 5 μ thick were taken at intervals of 1,200 μ from both parts (D and L) of the serial-sectioned organ. The number of intermediate, unexamined sections of the same thickness was thus 239. The number of sections (l) taken in this way for quantitative analysis amounted to an average 12 per animal, with a range of 7 to 25. The sections were projected in the dark room on a horizontal surface in a projection apparatus for low-grade magnification (Aristophot Leitz with Milar objective, F = 100 mm). Using a linear enlargement of 12 ×, the whole area of the section

being contained in *one* visual field, the outlines of all the parenchymal areas present in the section were drawn. Adipose tissue, lymphatic tissue, connective tissue, large blood vessels, and large ducts lying extralobularly were thus excluded.

The parenchymal areas thus drawn on paper were measured with an Ott planimeter. The total magnified parenchymal area in a section was denoted as P and given in mm². The real parenchymal area was denoted as p, and was thus $P : 12^2$. By addition of the P values for the individual sections, a total P value was obtained both for the D and L parts separately, and for the whole organ. The corresponding p values for D, L and D+L were calculated from these values. Since the interval between the sections was 1,200 μ, a conception could be obtained of the total parenchymal volume, V_p, *i. e.*,

$$V_p = p \times 1.2 \text{ mm}^3$$

The parenchymal volume was given both in absolute figures, V_p, and calculated per kg of body weight, V_p/BW.

Size of Islet Tissue

A calculation was made of the average islet size, on the basis of measurement of the cut surface of a sample of about 200 islets (in groups I A and IV B about 600 islets) from each animal. Here as well, use was made of the projection procedure. Using a linear enlargement of 320 ×, the microscopic picture was projected in the dark room on a horizontal surface. The microscope was a Leitz Dialux, with a mechanical stage and inclined ocular tube with attached projection mirror, and eyepiece with square diaphragm. In the apparatus used, the angle of the tube to the vertical plane (135°) could not be altered; this also applied to the reflection angle of the mirror (60°). Consequently, in order to obtain depiction at right angles on projection, it was necessary to let the microscope stand form an angle of 15° to the table; this was managed by using an oblique stand that

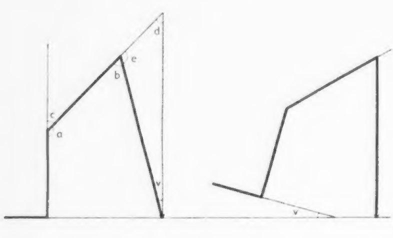

Fig. 8. Schematic diagram of passage of light in microprojection, with and without use of oblique microscope stand.

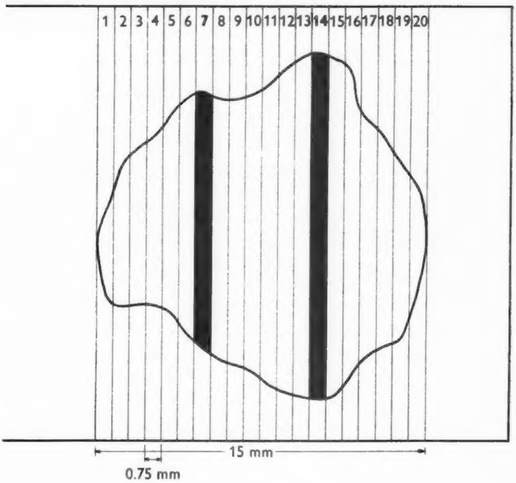

Fig. 9. Example of selection of an islet sample for measurement of islet size.

could be raised and lowered. (This is illustrated by the sketch in Fig. 8, in which angle $a = 135°$, angle $b = 60°$ and, therefore, angle c = angle $d = 45°$, angle $e = 120°$ and angle $v = 15°$.) Before each projection, angle v was checked; the degree of magnification was also checked with a stage micrometer.

The sample of the islets to be used for measurement of the size was taken as follows. Two equidistant, transverse strips the width of a visual field were taken from every or every other section (Fig. 9). Since the width of a visual field was 0.75 mm, a section 15 mm in diameter contained 20 such strips. Strips 7 and 14 were then chosen as being equidistant. Since the diameter of the sections ranged from about 12 to 18 mm, the strips chosen for measurement of the islet size varied from one section to the next. For example, when the diameter of the section was 12 mm, strips 5 and 11 were chosen; when it was 18 mm, strips 8 and 16 were used. The greatest diameter parallel to the longitudinal direction of the slide was always taken as the diameter of the section.

In each strip taken, the outline of all the islets lying entirely within the strip was drawn, as well as the islets of which some part lay beyond its right border. Islets lying partly beyond the left border were regarded as not belonging to the strip in question, and were not drawn. If the total number of islets in one strip amounted to less than 10, the strip directly to the right of it was included in the sample. If the number of islets was still less than 10 which, however, was relatively seldom the case, the strip directly to

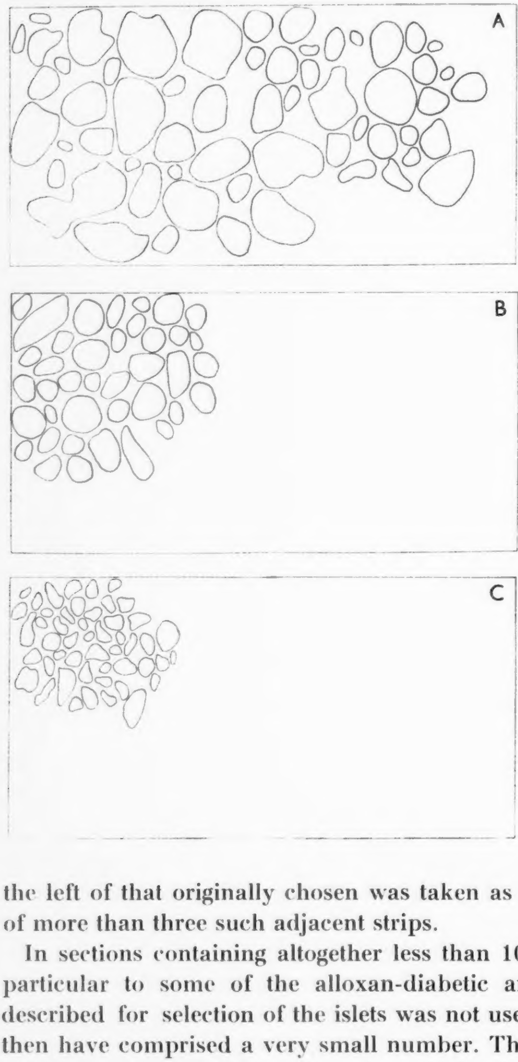

Fig. 10. Three examples of varying islet size in the islet sample.

A. *R. 225 (group IV F a).*
$I = 36{,}525\ mm^2$,
$N = 58$,
$M = 629.7\ mm^2$,
$m_i = 6{,}149\ \mu^2$.
B. *R. 157 (group IV C).*
$I = 12{,}460\ mm^2$,
$N = 39$,
$M = 319.5\ mm^2$,
$m_i = 3{,}120\ \mu^2$.
C. *R. 243 (group IV G b).*
$I = 5{,}870\ mm^2$,
$N = 49$,
$M = 119.8\ mm^2$,
$m_i = 1{,}170\ \mu^2$.

the left of that originally chosen was taken as well. No sample consisted of more than three such adjacent strips.

In sections containing altogether less than 100 islets, which applied in particular to some of the alloxan-diabetic animals, the principle just described for selection of the islets was not used, since the sample would then have comprised a very small number. The following procedure was used instead. When the total number of islets in the section was 60 to 100, every third strip was included in the sample; when the number was less than 60, every other strip was taken.

Using the method described, each sample comprised at least 20 to 30 islets from each section. This number was denoted as N. The area of these

drawn islets was measured with a planimeter, and the total measured islet area in a section was denoted as I and was given in mm². A mean value of the islet area, M, could then be obtained from the formula:

$$M = \frac{I}{N} \text{ mm}^2$$

The number of sections in which the islet area was measured was denoted as k.

Fig. 10 shows the results of measurements of the islet size in three separate cases, each representing the islet sample from a single section. *A* is illustrative of an animal with very large islets, *B* of an animal with islets of "normal" size, and *C* of an animal with fairly small islets.

The total number of islets in each section (1,200 μ intervals) was determined by systematic counting in a binocular microscope with a mechanical stage and square ocular diaphragms, at an enlargement of about 100 × (periplane eyepiece 8 ×, objective 13 ×, aperture 0.40). Starting at the left margin of the section, the islets were counted in each strip in succession. The same principle was then observed as in drawing the islets, *i. e.*, the islets lying partly outside the right border of the visual field were included, but not those partly outside its left border. This prevented any islet being counted more than once. The total number of islets in one section was denoted as n_i.

On the basis of the values of M and n_i thus obtained, the total islet area in one section, I_c, was calculated with the formula

$$I_c = M \times n_i \text{ mm}^2$$

In those cases in which a value of M was lacking for a particular section, since measurement of the islet size was often made in every other section only, I_c was calculated on the basis of the M value in the adjacent section.

By addition of the I_c values for the individual sections, a total I_c value was obtained for D and L separately, as well as for the whole organ, D + L. Since the linear magnification was 320 ×, a true value for the islet area, *i*, was obtained from the formula

$$i = S(I_c) : 320^2$$

Since the interval between the sections was 1,200 μ, a conception of the islet volume, V_i, could be obtained from the formula

$$V_i = i \times 1.2 \text{ mm}^3$$

The islet volume was also calculated per kg of body weight, V_i/BW.

A measure of the mean islet area in an individual animal, m_i, was obtained from the formula

$$m_i = i : S(n_i)$$

and was given in μ^2.

The total number of counted islets in an individual animal, $S(n_i)$, has also been given per kg of body weight, n_i/BW.

Table 4 comprises an example of the micromorphologic analyses of the size of the total pancreas parenchyma and of the size of the islet tissue in one case (R. 242, group IV A). On the grounds of space, it has not been possible to give an equally detailed account in every case. The summarized data for the individual animals in the different groups are, however, recorded in Tables II: 1 to II: 15 and Tables III: 1 to III: 15 (Appendix).

Alpha and Beta Cell Count

A differential count was made of at least 1,000 islet cells from each animal. Since the size of the islets varied in the different groups, this number was distributed over a varying number of islets, *i. e.*,

In non-diabetic groups with functioning exocrine parenchyma: 50 islets per animal;

In diabetic groups with functioning exocrine parenchyma: 75—100 islets per animal;

In group III, without functioning exocrine parenchyma: 110—150 islets per animal.

As a rule, the count comprised 25 islets in each section, sections being taken from both the D and the L part of the pancreas. Sections were taken which lay centrally in the relevant part of the organ, and which exhibited satisfactory Gomori staining for the purposes of a differential count. The count was made at a magnification of about 310 × (periplane eyepiece 10 ×, objective 25 ×, aperture 0.50).

A transverse strip (*cf.* Fig. 9) lying centrally in the section was sought, and a differential count was made of each of the islets in it in succession, until 25 had been counted. If less than 25 islets were present in one strip, the count was continued in every other strip to the right of it, until 25 islets had been counted. Only islet cells in which the nucleus had been cut through were included, but no regard was paid to the size of the nuclear fragment.

The results have been given as (a) the number of alpha cells, (b) the number of beta cells, (c) the total number of cells counted and (d) the

TABLE 4

Example of microscopic analysis of pancreas volume and islet tissue in one animal

	l	P	p	V_p	V_p/BW	k	I	N	M	n_i	I_c	i	V_i	V_i/BW
D	1	3,775								52	16,630			
	2	9,112				1	10,555	33	319.8	144	46,051			
	3	11,835								181	71,368			
	4	12,105				2	9,070	23	394.3	97	38,247			
	5	12,440								126	42,840			
	6	13,563				3	7,480	22	340.0	100	34,000			
	7	13,990								117	29,601			
	8	7,466				4	3,795	15	253.0	44	11,132			
	9	4,340								43	10,879			
S (D)	9	88,626	615.5	738.6	225.2	4	30,900	93	332.7	904	300,748	2.94	3.53	1.08
L	1	9,892								195	75,992			
	2	10,562				1	20,265	52	389.7	214	83,396			
	3	12,230								285	89,006			
	4	11,969				2	14,990	48	312.3	258	80,573			
	5	10,870								117	40,739			
	6	13,642				3	12,885	37	348.2	191	66,506			
	7	14,993								152	38,623			
	8	6,492				4	4,065	16	254.1	24	6,098			
	9	6,085								61	15,500			
S (L)	9	96,735	671.8	806.2	245.8	4	52,205	153	331.6	1,497	496,433	4.85	5.82	1.77
D + L	18	185,361	1,287.2	1,544.6	470.9	8	83,105	246	332.0	2,401	797,181	7.78	9.34	2.85

R. 242. Group IV A. BW 3.28 kg, PW 4.30 g (D 2.24 g, L 2.06 g). PW/BW 1.31 g, n_i/BW 732, m_i 3,240 μ^2

incidence of alpha cells in per cent. The results of the alpha and beta cell count in the individual animals in the different groups are assembled in Tables IV: 1 to IV: 15 (Appendix).

STATISTICAL METHODS

The statistical analyses were made according to current principles (BERNSTEIN & WEATHERALL; BONNIER & TEDIN; ERÄNKÖ). Thus, the following symbols and methods of calculation were used:

x = individual observation in a sample
n = number of observations in a sample
S = summation
$\bar{x}$ = arithmetic mean of the x values
s = standard deviation of a sample:

$$s = \sqrt{\frac{S(x-)x)^2}{n-1}}$$

$e(x)$ = standard error of the mean:

$$e(\bar{x}) = \frac{s}{\sqrt{n}}$$

df = degrees of freedom
$\bar{x} \pm t \times e(x)$ = fiducial limits of the mean
$(x_1 - \bar{x}_2)$ = difference between two means
$e(x_1 - x_2)$ = error of the difference between two means:

$$e(\bar{x}_1 - x_2) = \sqrt{\frac{S(x_1 - x_1)^2 + S(x_2 - x_2)^2}{n_1 + n_2 - 2}\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}$$

In comparing two mean values, the *t* test was used:

$$t = \frac{\bar{x}_1 - \bar{x}_2}{e(\bar{x}_1 - \bar{x}_2)} \text{ with df} = (n_1 + n_2 - 2)$$

A difference has been regarded as

probably significant at $P \sim 0.05$*
significant at $P \sim 0.01$**
highly significant at $P \sim 0.001$***

When comparing several groups collectively with other similarly collected groups, the *t* test was used as follows:

The difference $z = \dfrac{S\,x_1 + S\,x_2}{n_1 + n_2} - \dfrac{S\,x_3 + S\,x_4}{n_3 + n_4}$

The error of $z = e(z) = \sqrt{\dfrac{S\,[S(x-\bar{x})^2]}{S\,n-4}\left(\dfrac{1}{n_1+n_2} + \dfrac{1}{n_3+n_4}\right)}$

and $t = \dfrac{z}{e(z)}$ with df $= (S\,n-4)$

d = difference between two individual observations in two series of samples

$\bar{d}$ = mean of all the d values

s_d = standard deviation of the sample of differences

$e(\bar{d})$ = standard error of $\bar{d}$:

$$e(\bar{d}) = \frac{s_d}{\sqrt{n}}$$

The significance of $\bar{d}$ was evaluated with the *t* test, in which

$$t = \frac{\bar{d}}{e(\bar{d})} \text{ with df } = (n-1)$$

In some cases, the significance of a difference between two samples was determined according to the ranking method of WILCOXON:

$$t_{\infty} = \frac{T - n_1 \dfrac{n_1 + n_2 + 1}{2}}{\sqrt{\dfrac{n_1 \cdot n_2 \cdot (n_1 + n_2 + 1)}{12}}}$$

in which T = the rank total of sample n_1.

Some of the methods used were checked by means of double determinations. From these, the error of a single determination was calculated as:

$$\sqrt{\frac{S\,x^2}{2\,n}}$$

r = correlation coefficient of two variables x and y:

$$r = \frac{S(x-\bar{x}) \cdot (y-\bar{y})}{\sqrt{S(x-\bar{x})^2 \cdot S(y-\bar{y})^2}}$$

The significance of r was tested from the equation:

$$t = r\sqrt{\frac{n-2}{1-r^2}} \text{ with df} = (n-2)$$

b = regression coefficient of y on x:

$$b = \frac{S(x-x)\ (y-\bar{y})}{S(x-x)^{\ 2}}$$

The regression line was calculated from the equation:

$$y = y + b(x-x)$$

CHAPTER 4

DISCUSSION OF THE METHODS

FEEDING

In an investigation of the glucose tolerance and the morphology of the pancreas, the diet of the experimental animals is obviously of great importance. In the present investigation, the rabbits were fed on natural foodstuffs, and the quantity given per day was constant throughout the experimental period. It is true that the size of the portions was, after the initial period, estimated and not weighed, but the deviation from the measured amounts cannot have been large. It is evident that a synthetic diet of known composition is easier to standardize, and gives a more accurate picture of the animal's nutritional state, than does one composed of natural foodstuffs. However, it is apparent from the publications of FARRIS, HOGAN, LOOSLI and NORDFELT, among others, that the general condition, growth and fertility are optimal in rabbits only when they are fed on natural foodstuffs. This is the reason why I chose such a diet for the present investigation.

I calculated the quantity of digestible substances in the diet in question from tables giving the composition and nutritional value of different fodder*. The daily portion was found to contain approximately the following amounts: 15 g of protein, 5 g of fat, 80 g of nitrogen-free extract and 20 g of crude fibre. The daily caloric content amounted to approximately 480 calories.

On the basis of the investigations made by LEE, the basal metabolism of adult rabbits in the weight class 2.5 to 3.5 kg can be given approximately as 150 calories/24 hrs (*cf.* ALBRITTON). Even if the increase in this basic value due to activity is taken into account, the caloric content (480) might seem to be fairly high. Consequently, there might have been some degree of overfeeding. The following facts nevertheless argue against this supposition. (1) The animals were always hungry at feeding time. (2) No

* Issued by the National Animal Experimental Station of the Royal Agricultural College of Sweden.

excessive fat deposits were observed at autopsy. (3) In the four untreated controls in group IV A, the increase in weight amounted to 11.3 g/24 hrs during an average observation period of 40 days. In an additional 18 rabbits, which belonged to other groups but were under observation for an average 39.2 days before the experiment was started, the increase in weight was 11.2 g/24 hrs.—These figures are no higher than those stated to be normal in rabbits. Thus, the increase in weight in rabbits belonging to the weight groups in question can be calculated from the publications of EDIN, of JARL and of JOHANSSON *et al.* to be 11 to 15 g/24 hrs.

Finally, it can be pointed out that, even if a slight degree of overfeeding was nevertheless present, this factor was the same for all the animals. Consequently, it does not invalidate a comparison between the results in the different groups. Groups I and II form an exception but, as stated earlier (p. 35), underfed animals were used as special controls for these groups.

DETERMINATION OF BODY SIZE

The formula used to determine the body size ($BS = 56.33 \times BW^{0.436}$) is an empiric modification of the MEEH formula ($BS = k \times BW^{2/3}$). It differs from the corresponding formula of DUBOIS & DUBOIS for human subjects in that the height is not taken into account. However, as shown by BRODY *et al.*, this factor can be neglected in determinations of the body size in four-footed animals.

In determining the size of the pancreas and of the islet tissue, the results were correlated both to the body weight and to the body surface. In both cases, approximately the same correlation was obtained. TEJNING reported similar results with respect to the relation between the size of the islet tissue and the body size in rats. It may also be mentioned by way of comparison that HAIST & BELL found a good correlation between the insulin content of the pancreas and the body weight in rats. LEE, who studied the basal metabolism of the rabbit, found that it was even better correlated to the body weight than to the body surface. For these reasons, the size of the pancreas and of the islet tissue is given in the tables in relation to the body weight, but not to the body surface.

URINALYSES

Glucose.—Traces of reducing substances were found normally in all the rabbits, but never exceeded 0.5 per cent or 0.5 g/24 hrs, as expressed in

the Benedict test. Consequently, there was no difficulty in distinguishing this glycosuria from that appearing in alloxan diabetes.

Ketone bodies.—A comparison between the rapid method used (p. 39) and tests performed in the usual way with a fluid reagent showed good agreement. Thus, in a series of 137 unselected urine samples from human subjects, 53 were positive and 84 negative with the powder test, the corresponding figures being 48 and 89 when a fluid reagent was used.

Diastase.—WOHLGEMUTH's method seems to be less reliable than that of NØRBY (*cf.* LAGERLÖF). My reason for choosing it was that, at the time, it was the routine method in use at our clinic. Moreover, my object was only to obtain a rough idea of the rate at which atrophy of the exocrine pancreatic parenchyma takes place after ligation of the pancreatic duct.

BLOOD SUGAR DETERMINATIONS

Two blood samples were taken on each occasion, and the error of a single determination could be calculated on the basis of these double determinations. Since this error of the method might have varied at different blood sugar levels, the error was first calculated within three such levels. The following results were obtained with HAGEDORN-JENSEN's method.

In a blood sugar region corresponding to 70—130 mg/100 ml, the error of the method was $\pm$ 2.14 mg/100 ml (n = 54); the corresponding figure in the region 131—180 mg/100 ml was $\pm$ 1.88 mg/100 ml (n = 68), and in the region 181—250 mg/100 ml it was $\pm$ 2.80 mg/100 ml (n = 60). There was thus no significant difference between the magnitude of the error at these three levels. The error of the method could therefore be calculated irrespective of the blood sugar level. It was found to be $\pm$ 2.29 mg/100 ml (n = 182). No systematic difference was present between the first and the second sample in the double determinations at any of the blood sugar levels in question.

In many cases the blood sugar was determined in animals with jaundice (groups I and II). The possible influence of jaundice on the blood sugar determinations was therefore investigated in a series of samples; both icteric sera, and bilirubin solutions prepared *in vitro* were then added to glucose solutions of known strength. No significant effect was noted.

EVALUATION OF GLUCOSE TOLERANCE TEST

Previous Methods

Many different criteria have been used to evaluate the glucose tolerance test (*cf.* MOSENTHAL & BARRY). When the test is used for clinical diagnostic purposes, one or several of the following values usually form the basis for the evaluation: the fasting value, the peak value, and the time for returning to the fasting level. The last-mentioned factor has generally been regarded as of greatest consequence (CRAWFORD; LOZNER *et al.*; TUNBRIDGE & ALLIBONE). GOLDBERG & LUFT studied the glucose tolerance both in normal subjects and in certain endocrine disorders by means of three tolerance tests: the oral one-dose test, the oral one-hour two-dose test, and the intravenous test. The curves were evaluated on the basis of their deviation in σ units from the average curves of healthy subjects. Particular importance was attached to the following: in the oral one-dose test, the fasting value, the maximum value and the time for return to the fasting value + 10 mg/100 ml; in the oral one-hour two-dose test, the blood sugar at 0, 30 and 60 minutes; and in the intravenous test, the blood sugar at 0, 45 and 90 minutes, and the time for return to the fasting value.

AMATUZIO *et al.*, as well as CONARD *et al.*, analyzed the intravenous tolerance curve in an essentially different way. According to these authors, the tolerance curve is in agreement with an exponential function of the type $y = a \times e^{-kx}$, in which y is the blood sugar value at time x, and a is the extrapolated blood sugar value at time 0, expressed in mg/100 ml in excess of the fasting blood glucose. The constant k is defined as "the disappearance rate of glucose from the blood" (AMATUZIO *et al.*) or "le coefficient d'assimilation glucidique" (CONARD *et al.*). On the basis of these calculations, the authors concluded that k is constant and reproducible, and that it permits a definite differentiation between normal and diabetic types of curves. Consequently, it is preferable to "interprétations plus ou moins subjectives et incomplètes" (CONARD *et al.*). It is, however, found that the k value given by CONARD *et al.* as a normal value is of an entirely different order of magnitude to that given by AMATUZIO *et al.* Actually, it is in better agreement with the k value found by the latter authors in subjects with mild diabetes. This fact arouses doubts regarding the practicability of this method of calculation.

Another, simpler method was described by HIMSWORTH. The area covered by the part of the blood sugar curve above the level of the normal value is used as the basis of evaluation. According to this author, this area exhibits fewer individual variations than do the individual points on the

curve. The tolerance area is measured with a planimeter and expressed in "*milligram-minutes*". The same method was used by Ross & Tonks. According to them, the normal intravenous glucose tolerance test is represented by an area that does not usually exceed 3,500 mg-min, if its baseline ends at the 60-minute value. A similar method was earlier described by Jörgensen, but he added to the value of the area delimited by the blood sugar curve ("the area figure") the number representing the time from the end of the injection of glucose until the blood sugar had returned to the fasting level. He denoted the sum of these two figures as "the loading figure". Both Crawford and Tunbridge & Allibone have criticized this procedure, seemingly with good reason, the addition of a surface measure and a time measure being somewhat artificial, especially as the time factor is already included in the surface measure.

Present Method

The glucose tolerance area as calculated in the present investigation (p. 40), does not differ from the area delimited by the real blood sugar curve if the enlargement of the area implied by increasing the breadth of the outermost rectangles is taken into account. This is because the triangles formed by the intersection of the blood sugar curve and the upper borderlines of the rectangles (△ ABC and △ CDE in Fig. 1) are congruent.

The following comparison was made to test the reliability of the method. The tolerance area was calculated from five glucose tolerance curves, lying within five different blood sugar levels, both by planimetry and with the method of calculation described on page 40. The results were as follows:

Planimetric area mg-min	Calculated area mg-min	Difference mg-min
1,688	1,725	+ 37
4,295	4,265	— 30
4,775	4,755	— 20
5,273	5,270	— 3
8,405	8,385	— 20

A similar comparison was made between tolerance areas reported by Himsworth in a study of the influence of seven different diets on the glucose tolerance. The following figures were recorded:

Diet no.	Planimetric area mg-min	Calculated area mg-min	Difference mg-min
1	6,833	6,790	— 43
2	3,785	3,760	— 25
3	3,560	3,480	— 80
4	2,850	2,840	— 10
5	2,767	2,780	+ 13
6	1,815	1,770	— 45
7	1,136	1,190	+ 54

The values for the planimetric area are those given by HIMSWORTH. The calculated values are based on the blood sugar values approximated from his Fig. 2, since the exact figures were not given in tabular form. It may be inferred that the differences between the values obtained with the two methods are inappreciable in relation to the total tolerance area.

ALLOXAN ADMINISTRATION

As stated earlier, two types of alloxan dosage were used (p. 36). The reason was that a single dose of 200 mg/kg of body weight led to a considerably higher incidence of toxic damage than did a dose of 100 mg/kg given on two successive days. A detailed account of the findings is given in the relevant animal records. Briefly, it can be mentioned that, during the initial diabetic stage, acetonuria appeared in 61.1 per cent ($^{11}/_{18}$) and proteinuria in 100 per cent ($^{11}/_{11}$) of the rabbits given 200 mg/kg of alloxan in a single dose. In the same group, the primary mortality from renal or hepatic damage or diabetic acidosis amounted to 31.6 per cent ($^{6}/_{19}$). In a corresponding group of 16 animals given 100 mg/kg on two successive days, the incidence of acetonuria and proteinuria was 12.5 and 75.0 per cent, respectively, and the primary mortality 0 per cent.

The diabetogenic effect was, on the other hand, the same with both types of alloxan dosage (*cf.* Table 22). Moreover, once the initial toxic symptoms had disappeared after a few days, no difference was present between the two groups with respect to the excretion of glucose, the insulin requirement or the general condition.

The animals were starved for 18 to 20 hours before administration of alloxan. This was because the diabetogenic effect has been found to be more constant when the substance is administered to starved than to fed animals (KASS & WAISBREN; DE MOOR). Glucose was given as a protection

against the characteristic hypoglycaemia that is one phase of the triphasic blood sugar change following alloxan administration (see review by LUKENS). Consequently, I lost no animals on account of early hypoglycaemia.

The animals were regarded as diabetic as soon as they exhibited massive glycosuria, *i. e.*, when the Benedict test showed glycosuria of more than 3 per cent. This was almost invariably associated with considerable polyuria (500 to 1,000 ml/24 hrs, as compared with the normal 100 to 300 ml), and increased thirst.

Although it is possible to keep alloxan-diabetic animals alive for long periods without insulin therapy, insulin was given in the present investigation for the following reasons. Without insulin, there would have been some loss of animals in acidosis. Consequently, the animals remaining for the final histologic analysis would have consisted of a selection of cases with more or less mild diabetes. Furthermore, it is possible with insulin to keep the animals free from acidosis and, therefore, in good general condition with a normal appetite. The factor of nutrition will thus be the same in both the diabetic and the non-diabetic groups. The risks associated with operation are also less in insulin-treated animals. Finally, it has been pointed out by DE MOOR that initial insulin therapy prevents regenerative processes in the pancreas, and contributes to establishing a permanent diabetic condition.

BILIARY FISTULA TECHNIQUE

Previous Methods

Biliary fistula experiments have earlier been performed in a relatively large number of investigations, although in only a few of them in rabbits. Two different types of method have been used. In one of them, an *external* fistula is made; the bile is then led directly out of the body. In the other, the fistula is *internal*, the bile flow being diverted to some other part of the body than the duodenum, *e. g.* the genitourinary tract or the colon.

In *dogs*, the external route was used by ROUS & MCMASTER (1923). According to these authors, it had not earlier been possible to make a biliary fistula function for more than a few days, owing to imperfect technique. They found that if the drainage tube from the common bile duct was allowed to run in a relatively long (10 to 12 cm) U-shaped loop intra-abdominally, the animals could be kept alive for several months. Closest to the common duct they used a short glass cannula, joined to a

soft rubber tube, which was connected by a U-shaped glass tube to a stiffer rubber tube; on passage through the abdominal wall, this was able to withstand any muscular contractions. The omentum formed a connective tissue capsule around the drainage tube. The bile was collected in a rubber balloon, kept in a rattan basket attached to the side of the animal.

DRAGSTEDT & WOODBURY used a simpler technique, and passed the drainage tube by the shortest route from the gallbladder to the abdominal wall. They did not state how long such a fistula could be made to function. The same method was, however, used by BISSELL & ANDREWS in their thorough analysis of the problems associated with biliary fistulas, and they were able to keep dogs alive for 4 to 5 months with its use. A similar method was applied, also in dogs, by LERICHE & JUNG and by VERNE. Internal fistula methods in the dog have been described by KAPSINOW *et al.* and by PEARCE & EISENBREY. The former sewed the common duct end-to-end with the ureter, after performing unilateral nephrectomy. The latter first ligated the common duct, and then sewed the gallbladder into the renal parenchyma, opened on its convex aspect.

In the *rat*, an external fistula technique has been reported by SAWYER & LEPKOVSKY. These workers led the bile from the common duct into a small glass flask, which lay loose in the abdominal cavity; it was attached to a drainage tube through the abdominal wall for emptying the collected bile. This method was later modified by COLWELL to allow passage of bile alone, since with SAWYER & LEPKOVSKY's technique, the fistula gave a mixture of bile and pancreatic juice, owing to the fact that in the rat pancreatic ducts open into the common bile duct. BORGSTRÖM collected a mixture of bile and pancreatic juice from rats by direct diversion from the common duct through the abdominal wall to the right side of the animal, in combination with the activity-limiting cage described by BOLLMAN.

Internal fistula methods have also been described in the *rat*. GREAVES & SCHMIDT anastomosed the bile duct close to the liver with the descending colon by means of a silver cannula; they were able to keep the animals alive for 3 to 4 weeks. HARRINGTON *et al.* made a similar anastomosis between the bile duct and the vas deferens in male rats; their animals survived for 3 to 4 months. FRIEDMAN *et al.* described a still more drastic method. The common duct was canniculated with polyethylene tubing, which was passed through the left leg, to emerge from the foot pad. In a study of the effect of ligation of the pancreatic ducts in rats, CLOWES & MACPHERSON anastomosed the bile duct proximal to the entry of the pancreatic ducts with a jejunal loop, using a short polyethylene tube.

Biliary fistula experiments in the *rabbit* have been reported by HALPERT.

A glass cannula was introduced into the common bile duct close to the duodenum. In these experiments the observation time was, however, only a few hours.

Present Method

At the beginning of the investigation, I believed that the method of ROUS & MCMASTER would be the most suitable. However, I soon found that the intervention was facilitated technically if polyethylene tubing was used closest to the gallbladder, instead of a glass cannula. I also found it better to make the U-shaped portion of the drainage system shorter than was suggested by ROUS & MCMASTER, particularly since in some cases a long loop had caused fatal strangulation ileus. I nevertheless adhered to the basic principle of their method, *i. e.*, a relatively long drainage system, although the greater part of it lay outside the abdominal cavity.

I also found, in a preliminary investigation, that several animals died in a syndrome of dehydration and cachexia. The same finding was made by SAWYER & LEPKOVSKY in rats with a biliary fistula, whereas the survival time was longer with ligation of the common duct only, or when there was a fistula but its outlet was occluded. According to these authors, such results indicate "the indispensability of the bile". The same view was held by COLWELL, who suggested that the bile plays a greater role in regulation of the fluid and electrolyte balance than in digestion and absorption. He based his view on the fact that the survival time is far longer in rats with an internal biliary fistula, when absorption of fluid and electrolytes is possible, than with an external fistula. BISSELL & ANDREWS nevertheless expressed doubts regarding the possibility of a disturbance in the acid-base equilibrium with a biliary fistula, on the grounds that the electrolyte loss is so small that it can easily be compensated by the dietary intake. This view was, however, based on experiments on dogs, and it seems probable that in this respect dogs differ essentially from smaller experimental animals.

I accordingly decided to administer to the animals, by the parenteral route, approximately the same quantity of fluid and electrolytes as that lost with the fistular bile. Oral administration was out of the question, since the primary experimental requirement, *i. e.*, to render the gut completely free from bile or any of its components, would then have been unfulfilled. The composition of the replacement fluid used was based partly on analysis of fistular bile in some preliminary experiments, and partly on some earlier investigations (COOKE & CROWLEY; GAMBLE;

Ravdin *et al.*). There was only a slight difference between the electrolyte content of the replacement fluid and that actually found in the fistular bile of animals in group I A (Table 6).

AUTOPSY, HISTOLOGIC AND HISTOCHEMICAL TECHNIQUE

Autopsy

The reason for which I chose exsanguination for sacrificing the animals was that dissection of the pancreas from surrounding tissue is considerably easier in an organism from which the blood has been emptied.

Preparation of Pancreas

The anatomy of the rabbit pancreas has been known ever since Claude Bernard gave his classic description in 1856:

> "Chez le lapin le pancréas offre une apparence tout à fait différente de celle que nous avons signalée dans les animaux précédents; au lieu que sa substance soit ramassée dans un organe épais, elle est en quelque sorte étalée en arborisations très-délicates entre les feuillets du mésentère. Le conduit principal, qui résulte de la réunion successive en un tronc commun des différentes branches secondaires, descend en suivant parallèlement les vaisseaux sanguins qui sont compris dans l'anse duodénale, et vient s'ouvrir dans la portion ascendante du duodénum à une distance de l'orifice pylorique qui peut varier de 30 à 50 centimètres, suivant la taille du lapin... Il y a en outre une portion du pancréas qui se prolonge en forme de queue vers la rate, et qui est un peu plus épaisse que le reste de l'organe."

Jaffé distinguished between three portions of the pancreas in the rabbit: a splenic, a retroperitoneal and a duodenal portion. Owing to the diffuse nature of the gland, any such division is, however, to some extent artificial. For the purpose of the present investigation, I found it sufficient to divide the pancreas into a duodenal and a splenic part, as shown in Fig. 6. This provided pieces of tissue of a suitable size for fixation, with the organ packed into a relatively small volume. This division also permitted a separate evaluation of the morphologic conditions in two different parts of the organ. If the pancreas had been fixed in one piece, there would have been a risk of incomplete penetration by the fixative. This risk was further diminished by the preliminary 5-minute fixation. Care was taken to avoid compression of the organ, since this could have affected calculation of the volume. With the technique used, the pancreas was converted after fixation into two cylindric pieces with plane limiting surfaces, and each section consisted of a more or less circular portion of tissue with a dia-

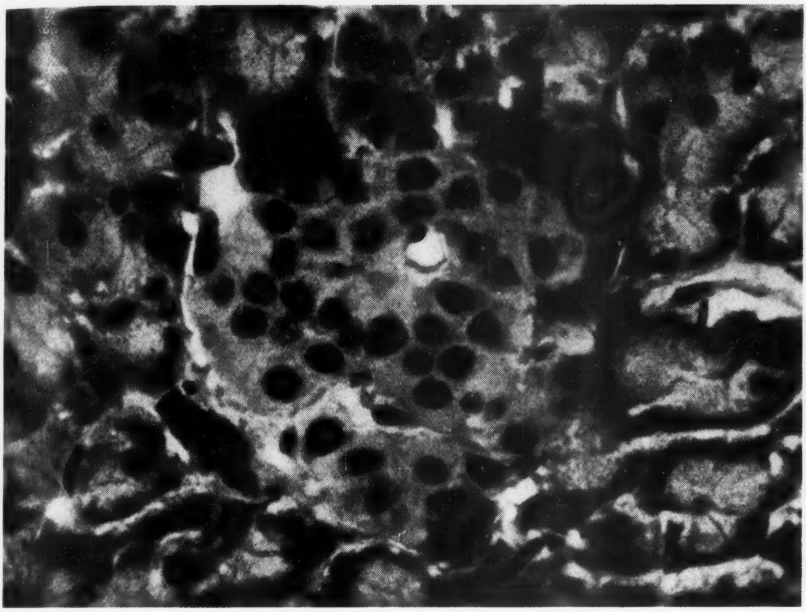

Fig. 11. Pancreas: untreated control (R. 242, group IV A). Marked granulation of the relatively few alpha cells in periphery of islet. Beta cells pale, with scarcely distinguishable granulation. Gomori stain. Magnification 730 X.

meter of about 15 mm, containing a varying proportion of pancreas parenchyma, adipose tissue, blood vessels and connective tissue.

The Gomori stain was chosen as being a generally accepted and relatively simple technique, which permits good differentiation between the alpha and beta cells of the islets. Moreover, in contrast to the silver method, it can be used in serial sectioning of paraffin-imbedded specimens (*cf.* Hultquist & Tegner).

In similarity to other granule-staining methods it is, however, to some extent variable with respect to the intensity of staining of the specific granules. In the present material, this applied chiefly to the granules of the beta cells. The red staining of the cytoplasm in the alpha cells was almost invariably distinct and homogeneous. The stainability of the beta cells, on the contrary, varied somewhat not only from one animal to the next, from one section to the next and often in the same section, but even in an individual islet. This variability was present to the same extent in

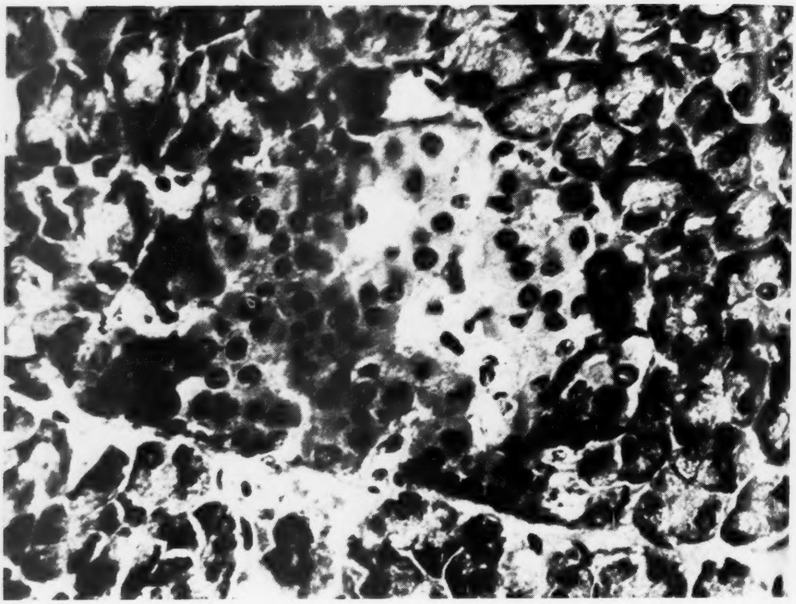

Fig. 12. Pancreas: untreated control (R. 178, group IV A). Variation in beta cell granulation; distinct granulation in half cut surface of islet, scarcely distinguishable granulation in other parts. Marked granulation of alpha cells in periphery of islet. Gomori stain. Magnification 500 X.

both experimental animals and controls. Figs. 11 to 13 illustrate this phenomenon. They all derive from animals in control group IV A. Thus, Fig. 11 shows beta cells with an extremely light cytoplasm and scarcely discernible granules, and Fig. 12 an islet in which half the cut surface contains similar beta cells almost devoid of granules, whereas the other half has beta cells with fairly deeply stained granules. Finally, in Fig. 13, all the beta cells exhibit a strongly and distinctly blue-granulated cytoplasm. This variation in the stainability of the beta cells did not, however, in any way affect the possibility of differentiating between them and the alpha cells.

The inhomogeneity of the beta cell granulation in Gomori-stained specimens has been pointed out earlier by Gomori and by Ferner. It is not possible to determine whether the variation is due to the technique, or whether it has a physiologic basis. For this reason, I made no attempt in the present investigation to draw any conclusions regarding the functional

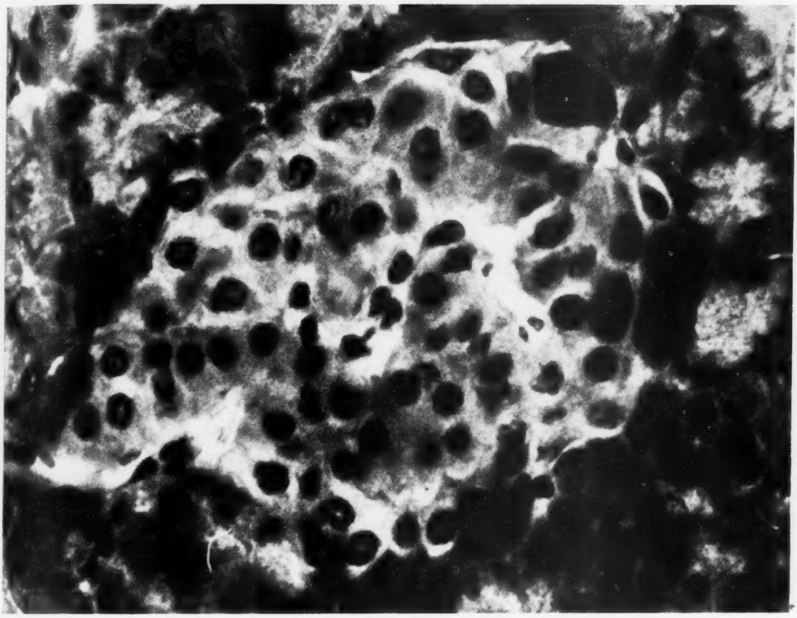

Fig. 13. Pancreas: untreated control (R. 178, group IV A). Distinct granulation of all visible alpha and beta cells in section. Gomori stain. Magnification 730 X.

state of the beta cells in the different animal groups on the basis of the degree of granulation of these cells, as has been done in the works of BARRON, of BELL and of HARTROFT & WRENSHALL, for example. In this connexion, mention may be made of BEST's experience, *i. e.*, that although as a rule there is a correlation between granule count and insulin content, there are situations in which such correlation does not exist.

Even if, in the present material, the variation in the beta cell granulation were actually an expression of a varying functional state in these cells, difficulties would be encountered in interpreting the observations. Degranulation may indicate either hyperfunction with immediate release of the secretion formed, or hypofunction with resulting low secretion. Analogously, a high granular content can be interpreted either as hyperfunction with resulting plentiful secretion in the cell, or as hypofunction with storage of the secretion. Comparison can be made with SELYE & STONE's discussion of the functional significance of a variation in the stainability of the lipid granules in the adrenal cortex.

Histochemical Technique

The glycogen, lipid and phosphatase methods were used only for qualitative evaluation, and the purpose of the numerical grading was merely to give a rough idea of the relative quantity of these compounds in the respective organs.

Since all the animals were killed at the same time of day, the possible influence of the rhythmic 24-hour changes in the liver glycogen was ruled out (FORSGREN; MÖLLERSTRÖM). Post mortem glycogenolysis has been discussed and studied by SWENSSON. In the rabbit liver, it was found to start 2.6 minutes after decapitation, and after 15 minutes only 84 $\pm$ 5 per cent of the glycogen content of a sample taken 40 seconds after death could be found in the liver. After the first 30 minutes, glycogenolysis proceeded more slowly. In my material, the interval between killing the animal and taking the glycogen sample was constant, and amounted to about 15 minutes. A certain degree of post mortem glycogenolysis must therefore have occurred. However, since this could be presumed to have affected all the animals to the same extent, I considered it justified to make semi-quantitative comparisons between the glycogen content in the liver of the respective animals. In several cases the glycogen method was tested by parallel staining with iodine methods and with saliva-treated controls.– In none of the cases did I observe the presence of intranuclear glycogen.

Glycogen could not be demonstrated in the cortical tubules of the kidney in any animal, diabetic or non-diabetic, contrary to the conditions in human diabetes (*cf.* WARREN & LECOMPTE). On the other hand, *large quantities of glycogen were found in the epithelium of the papillary ducts, close to their opening into the renal pelvis,* in practically all the animals, both experimental and control. Judging by the available literature, this has not earlier been the subject of comment, although it was mentioned by LUBARSCH in a brief note: "So gibt Best an, dass es bei Kaninchen schon normalerweise 'an der Papille' vorkommt". The significance of this phenomenon is unknown. It is of no importance in the present investigation.

The phosphatase technique was used in view of the importance of this enzyme in dephosphorylation of glucose in glycogenolysis, and the relation between the level of the blood sugar and the liver phosphatases shown by DRABKIN. Since plentiful alkaline phosphatase is normally present in the proximal tubules of the kidney, the reaction was applied in this organ as well, to check the technique.

As will subsequently be shown, this part of the investigation provided no information of value for the relevant problems. This was because large

quantities of the enzyme could be demonstrated at typical sites in the liver, kidney and duodenum in most cases, irrespective of the type of experiment performed. The reason may have been that I used a semi-quantitative technique only.

QUANTITATIVE MICROMORPHOLOGIC ANALYSES

Size of Total Pancreas Parenchyma

The method used for estimation of the total volume of the pancreas is in accordance with the generally accepted technique for micromorphologic volume determinations (*cf.* AGDUHR; CARLSON *et al.*; ENGFELDT; ERÄNKÖ; ROMEIS; TEJNING). It is particularly useful in a study of an organ of the type of the rabbit pancreas, in which the actual parenchyma is disseminated in a more or less abundant quantity of adipose tissue. However carefully the organ is dissected out, it is impossible to free it entirely from adipose and connective tissue. Consequently, the weight gives only an approximative idea of the real size of the parenchyma. It is true that in a comparison between normal animals in about the same nutritional state, the weight may be of certain relative value. The uncertainty increases when—as in the present investigation—a comparison is to be made between the weight of the pancreas in normal animals with a normal quantity of adipose tissue, and that in animals in which a more or less considerable reduction of the adipose tissue has occurred as a result of interference with the bile flow. In such cases, micromorphologic determination of the parenchymal volume is a necessary complement to weighing.

The accuracy of the former technique is highly dependent on the size of the interval between the sections studied. If it is exceedingly small or non-existent, it is no longer a question of estimation of the volume of the organ, but of direct measurement (ERÄNKÖ). However, on practical grounds, it is necessary in most cases to use a relatively large interval between the sections, except when the organ is very small. Otherwise, the method would be so laborious and time-consuming that it would be unfeasible to investigate more than a few animals.

The interval between the sections used in the present investigation, *i. e.*, 1,200 μ, was chosen partly with consideration to calculation of the islet tissue (*vide infra*), and partly in view of what I had found, in practice, to be feasible. With the method used for preparation of the organ in

connexion with fixation, the various sections were of about the same order of magnitude as far as the area was concerned. Consequently, it could be assumed that they were relatively representative samples of the parenchymal area.

Support for this assumption was given by investigation of two rabbits (group I A and IV B, respectively), in which the size of the total pancreas parenchyma was determined from sections taken at intervals of both 600 μ and 1,200 μ. The following results were obtained:

Animal no.	Interval betw. sections μ	l	P mm²	p mm²	V_p mm³	V_p/BW mm³
R. 101	600	21	487,451	3,385.1	2,031.1	949.1
R. 101	1,200	10	245,656	1,705.9	2,047.1	956.6
R. 102	600	19	282,126	1,959.2	1,175.5	511.1
R. 102	1,200	9	135,809	943.1	1,131.7	492.0

It may be inferred that the difference between the figures obtained with the two methods of determination is not particularly large, and does not influence the evaluation of the difference between the size of the total pancreas parenchyma in the respective animals.

The equation $V_p = p \times 1.2$ mm³ is based on the following simple arguments:

1. The parenchymal volume in a section 5 μ thick with a parenchymal area of p mm² is assumed to be $p \times 0.005$ mm³.

2. The magnitude of this volume is assumed to be representative of, and thus identical with, the parenchymal volume in 239 sections 5 μ thick, distributed on both sides of the section in question, *i. e.*, the volume is the same in all 240 sections in the 1,200 μ thick piece of tissue.

Consequently, the total volume of the parenchyma in this piece of tissue is:

$$(p_1 \times 0.005) + (p_2 \times 0.005) + \ldots .(p_{240} \times 0.005) =$$
$$p\,(240 \times 0.005) =$$
$$p \times 1.2 \text{ mm}^3$$

The planimeter method was chosen for measurement of the area, since it is considerably simpler and quicker than the older technique with cutting out and weighing of the areas drawn. Two identical planimeters were used by two assistants. The apparatuses were frequently tested against each other, and were found to give identical values. All planimetric measure-

ments were based on double determinations. The error of a single determination was calculated from these double determinations; it was found to be the same for both assistants, *i. e.*, $\pm$ 24 mm². Within the capacity of the apparatus, adjacent areas on the paper were as far as possible taken together in the measurements.

Size of Islet Tissue

Previous methods.—Ever since it became apparent, early in the present century, that the islets of Langerhans represent an independent organic system with endocrine functions, numerous methods have been published for determination of this tissue disseminated in the exocrine parenchyma. A detailed survey of these methods is beyond the scope of the present paper, particularly since they have been described and discussed in detail in several previous reviews, *e. g.* ARNDT & NEUMANN; BARGMANN; FERNER; KRAUS; TEJNING; WARREN & LECOMPTE. Mention can, however, be made of a few of the most important of these methods, as examples of different ways of approaching the problem.

There are two ways of investigating the islet tissue: (1) to determine the number of islets, and (2) to determine their size.—The total quantity of islet tissue in the pancreas can then be calculated from these data.

Fundamental investigations have been made by two workers in this field: HEIBERG and BENSLEY. HEIBERG determined both the number of islets per 50 mm² of parenchymal area in sections taken from different parts of the organ, and the islet size expressed as the maximum and the minimum diameter of the cut surface of a certain number of islets. Although this method of stating the islet tissue in relation to the exocrine parenchyma has been used by many workers, it is evident that it does not give satisfactory information when there are variations in the size of the exocrine tissue. Obviously, the method cannot be applied in a study of the islet tissue in exocrine atrophy, *e. g.* after ligation of the pancreatic duct.

On the basis of a similar criticism of HEIBERG's work, HAMMAR & HELLMAN introduced another method in a thorough study of the islet tissue in a child with "Thyreoaplasie". Representative sections were taken from four different parts of the pancreas; the area of the exocrine parenchyma was then determined at a magnification of 17 ×, and the islet area at a magnification of 100 ×. They used the technique well known in quantitative histology, *i. e.*, drawing the relevant tissues on standardized paper and then cutting out and weighing these pieces of paper. Since the absolute

weight of the organ was known, the weight of the endocrine parenchyma could then be determined separately.

A similar "paper method" for quantitative estimation of the pancreatic islet tissue in man at different ages has been described by OGILVIE. In a later publication (MACLEAN & OGILVIE), determination of the islet area was simplified by using a calibrated 0.5 mm squared grid inserted into the eyepiece of the microscope.

An important source of error in such determinations, that was already pointed out by BENSLEY, is the difficulty of obtaining sufficiently representative sections, since the distribution of islets—particularly as regards their number—varies greatly in different parts of the gland. In small experimental animals, in which examination can be made of a fairly large number of sections in relation to the size of the organ, this source of error can be eliminated to a varying extent. This is, however, impossible in a study of the pancreas of larger organisms, as for instance in the dog or in man.

BENSLEY's own method, which was applied to the guinea-pig pancreas, did not make use of cut sections. Instead, the total number of islets in the whole gland was counted directly in whole pieces of the organ, after *intra vitam* staining with neutral red, which stains the islets selectively, but leaves the remaining parenchyma transparent. With this method, the results are "...actually what they purport to be and not the result of computations of the total content made from partial counts" (BENSLEY 1914/15).

The neutral red technique was also used in a modified form by HAIST & PUGH in rats. After intravenous injection of a 2.5 per cent solution of the dye, the animals were killed; the pancreas was then dissected out and cut into "very small pieces". These pieces were compressed tightly between glass plates and examined in a microprojector at a magnification of about 80 ×. The area of the compressed islets was determined planimetrically, as was the total pancreas area. Using these values and the known weight of the pancreas, the size of the islet tissue was calculated with the help of a correction factor for compression of the organ. This method was used by HAIST *et al.* in an extensive study of different factors influencing the volume of the islets of Langerhans (*cf.* p. 30). In similarity to other authors, HAIST *et al.* stressed that the islet to acinar ratio is not a satisfactory gauge of changes in the volume of the islet tissue.

As far as I have been able to ascertain, there is only one account in the literature of the quantitative morphology of the pancreatic islets in the *rabbit*, *i. e.*, that of JAFFÉ. Using Bensley's neutral red method, he

determined the number and distribution of the islets in three different parts of the organ in 6 rabbits (*cf.* p. 68). With the same technique, he determined in 4 rabbits the maximum and minimum diameters of about 300 islets from each animal, the islet area being calculated in μ^2. The proportions of acinar and islet tissue were further determined with the "paper method" from serial sections stained according to Gomori. Finally, the number of cells per islet and the proportions of alpha and beta cells were determined in 257 islets from 8 animals. The total number of islets was found to vary between 47,720 and 89,810, from which data a mean of 61,970 can be calculated. The mean size of the islets was 6,160 μ^2 in the duodenal part, 10,480 μ^2 in the retroperitoneal, and 5,850 μ^2 in the splenic part.

The total number of islets is of about the same order of magnitude as that obtained in the present investigation, when calculating n_c with the help of FLODERUS' formula, but the average islet size is greater than that I found. The discrepancy may be due to the fact that I focused particular attention on the numerous small islets. Another reason may be differences in the material in the respective investigations.

A thorough analysis of the quantitative morphology of the pancreatic islets in the rat was published by TEJNING. The size of the islet tissue was determined with the aid of a volumetric and a numerical method. The former does not differ from the "paper method" already described. If the total area of all islets observed at a linear enlargement of 160 × in the organ serially sectioned at intervals of 300 μ is denoted as S, and the islet volume as V, the following equation is obtained:

$$V = \frac{S \times 0.3}{160^2} \text{ mm}^3$$

A similar volumetric method was used by HULTQUIST in a study of the pancreatic islets in the rat foetus.

The numerical method of TEJNING is based on a mathematical study by WICKSELL of the "corpuscle problem", which implies the following. When spherical or ellipsoid bodies of different size in an organ are severed by sectioning of the organ, the *apparent* distribution of small and large bodies on the cut surfaces does not correspond to their *real* distribution. This is because: (1) the apparent diameters of the bodies are usually less than the real diameters, since the chance of the corpuscles being cut exactly in the equatorial plane is relatively small, and (2) an arbitrary section through the organ contains a greater number of large bodies than of small, since the former are more often within the range of the plane of the section.

Consequently, such a section does not give a correct picture of the real distribution between large and small bodies.

In BENSLEY's method, in which the islets are counted in whole pieces of the organ, there is obviously no such "corpuscle problem". But it is unavoidable in all sectioning methods. Only few authors have taken this matter into account. HAMMAR & HELLMAN were aware of the problem and, in their cases, calculated empirically reduction and correction factors for determination of the real number of islets from the observed number. They stressed, however, that these factors have no generalized validity.

OGILVIE described a method for calculating the radius of the real average islet from the radius of the observed average islet, and thence of the volume of one "real" islet. The total number of islets was obtained by dividing the total islet volume calculated with the paper method by the volume of a "real" average islet. This method has been criticized by TEJNING.

With the help of the mathematical relation between the apparent and the real distribution of islets, as calculated by WICKSELL, TEJNING calculated the real number of islets in the organ from the observed number distributed in different size classes. It was then assumed that the islets are spherical or ellipsoid, and that they are evenly distributed in the organ. The latter assumption in particular implies a considerable approximation, since it is known that the density of the islets differs greatly in different parts of the organ. TEJNING's work also contains another source of error, which presumably cannot be disregarded. Islets with a diameter of less than 47 μ, *i. e.*, with a surface of less than 1,734 μ^2, were not included in the calculations. As TEJNING himself showed, the numerical distribution curve of the islets is excessively asymmetrical, with a considerable predominance of the small and smallest islets. Consequently, exclusion of those less than 1,734 μ^2 might introduce a not inappreciable error in calculation of the total number of islets in the organ. It may be mentioned by way of comparison that, in JAFFÉ's work, the smallest islet measured was 300 μ^2. In the present investigation, the corresponding figure was 293 μ^2 (see p. 83).

Finally, in TEJNING's method, although the gland was compressed between two slides with rubber strings during preparation, no correction was made for this compression factor in calculation of the size of the islet tissue, as was done for instance in HAIST & PUGH's aforementioned method.

Another solution of the corpuscle problem was suggested by FLODERUS in a quantitative micromorphologic analysis of the human hypophysis. In this case, the corpuscles consisted of cell nuclei. FLODERUS gave a simple

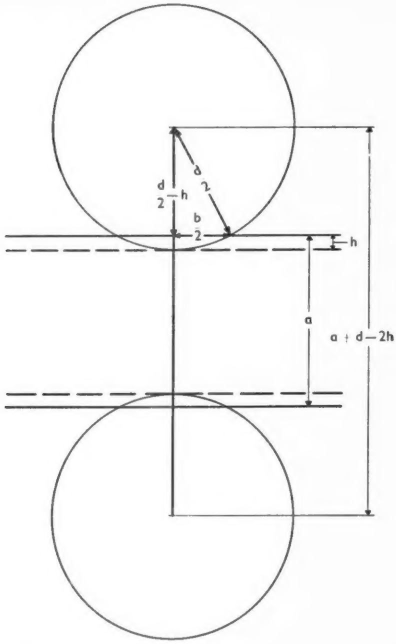

Fig. 14. Diagram for calculation of total number of bodies in an organ (according to FLODERUS*).*

reduction formula, with which it is possible, from the number of observed bodies in a section, many of which obviously do not have their centre within the section, to calculate the number of bodies that, in the strict sense really "belong" to the section, *i. e.*, have their centre within it. The derivation of FLODERUS' formula may be inferred from Fig. 14. If n denotes the number of observed bodies, a the thickness of the section, d the diameter of an average body, and h the height of the smallest observable segment of a body, the number of bodies with their centre within the section is obtained from the formula:

$$n \cdot \frac{a}{a + d - 2h}$$

The formula presupposes, as in that given by WICKSELL, that the bodies are spherical, that they are evenly distributed in the organ, and that they are of the same size. FLODERUS nevertheless stated that, in graphical control investigations, the formula gave equally reliable results with different sized bodies as with those of the same size, if d was calculated as a mean

value of the diameter of the bodies. BACKMAN found, however, that the formula yielded somewhat lower values when d comprised a common mean, than when the material was divided into size classes, and the number of bodies was computed separately in each class.

FLODERUS' formula has also been used by BLOCK in a quantitative morphologic investigation of the follicular system in women.

Present methods.—When choosing a method for estimation of the size of the islet tissue, I considered a volumetric method of the type used by TEJNING to be the most suitable. For measurements of area, I nevertheless regarded planimetry as preferable, since it is both quicker and more exact than the "paper method". As far as the interval between the sections was concerned, it was evident that an exact reconstruction, with examination of all the serial sections, was out of the question if more than a few animals were to be studied. Consequently, it appeared necessary to choose so large an interval between the sections that there could be no risk of the same islet being included more than once. It could be inferred from earlier investigations that the greatest islet diameter observed in different species of animals ranged from 300 to 500 μ (BARGMANN; JAFFÉ; KRAUS; TEJNING). I assumed that this would apply to rabbits as well, and therefore chose 600 μ as the interval between the sections.

However, I soon found that even with this interval, the number of sections per animal was so large that a complete analysis of the whole material was beyond the limits of possibility. Moreover, as in calculation of the size of the total pancreas parenchyma (V_p), I obtained largely the same results when the interval was increased to 1,200 μ. Consequently, the sections taken at this interval were regarded as containing, on the whole, representative samples of the islet tissue in the surrounding piece of tissue 2 by 600 μ. It is true that the islets in it were changed in shape, size and number, but their total cut surface was assumed to be constant. On the basis of this assumption, the equation

$$V_i = i \times 1.2 \text{ mm}^3$$

was obtained, analogously with $V_p = p \times 1.2 \text{ mm}^3$.

The total *number* of islets (n_i) in these sections could be established by systematic counting, as described earlier, but it was not possible to measure the *size* (M) of all these islets, since this would have implied drawing and planimetry of about 1,500 islets from each animal.

Consequently, it was necessary for measurement of the islet size to take as representative a sample as possible. The size of this sample was determined as follows.

In two groups (group I A and the corresponding control group IV B) each comprising 8 animals, the cut surface of 500 islets from each animal was drawn and measured. This number of islets was chosen as being the greatest number that could feasibly be measured in an individual case. The 500 islets were distributed in 4 sections (125 islets in each), 2 from the D part and 2 from the L part. These 125 islets comprised about two-thirds of the total number in each of the sections. Each section was examined strip by strip, beginning at the left margin, until 125 islets had been obtained.

The results were evaluated by means of a variance analysis. This provided a conception of the variance between the animals, between the two parts, between the sections and between the islets. The greatest variance was found between the islets, and the smallest between the parts. The latter was of such an order of magnitude that it could be neglected; this could be confirmed in the subsequent analysis of the material. It could also be established that the standard error of the mean in an individual animal was relatively large if less than 100 islets per animal were examined. It was considerably smaller on measurement of 100 to 200 islets per animal, and remained on the whole constant when more than 200 islets per animal were measured.

The variance in the total mean islet value for a group of animals, ε^2, could then be estimated by means of the formula

$$\varepsilon^2 = \frac{\sigma_1^2}{n_1} + \frac{\sigma_2^2}{n_1 \cdot n_2} + \frac{\sigma_3^2}{n_1 \cdot n_2 \cdot n_3}$$

in which σ_1^2 = variance between animals, σ_2^2 = variance between sections, σ_3^2 = variance between islets, n_1 = number of animals, n_2 = number of sections per animal (= k), and n_3 = number of islets per section (= N).

By taking $n_2 = 8$, and $n_3 = 25$, *i. e.*, 200 islets per animal, the following estimate of ε was obtained for different values of n_1.

n_1	ε	
	a μ^2	b μ^2
1	509	360
4	255	180
9	170	120
10	127	90

In this table, a is calculated on the basis of the values obtained in group I A, and b on the basis of those obtained in group IV B. The former represents a treated group of animals, and the latter an untreated normal group. Consequently, the figures given presumably represent the order of magnitude of the variability in the whole material.

On the basis of these results, a sample of about 200 islets per animal was fixed as being sufficiently large for evaluation of the islet size. It is scarcely practicable to measure less than 20 to 30 islets per section. In view of the number of sections per animal (l), it was therefore impossible to take islets from every section for the sample, as the number would then have been greatly in excess of 200. As a rule, islets from every other section only were measured; this is the reason why the number of such sections (k) is less than l. Since no systematic variation was found between the sections, either in the test described or in the material as a whole, this procedure can be presumed to have had no influence on the results.

On natural grounds, the number of islets measured per animal did not amount exactly to 200. Since my principle was to take all the islets present in entire strips, a sample from a section with a high islet content was necessarily larger than one from a section with few islets. A selection principle of this type is required in order to eliminate any subjective factors associated with sampling. Moreover, it ensures a relatively constant relation between the number of islets measured (N) and the number counted (n_i).

Obviously, the sum of the islets counted in the sections, $S(n_i)$, does not give the *total number of islets* in the pancreas. It can, however, be used satisfactorily as a figure for comparisons between the individual animals and between the different groups. I nevertheless considered it desirable to attempt a computation of the total number of islets in the whole organ. The method described by WICKSELL and applied by TEJNING could not be used, since no classification of the islets according to size had been made. A possibility was to apply the reduction formula given by FLODERUS (see p. 79 and Fig. 14).

The terms contained in this formula could be defined as follows:

$a = 5\ \mu$

d is obtained from the m_i value as $2\sqrt{\frac{m_i}{\pi}}$

h can be calculated from the equation

$$\left(\frac{b}{2}\right)^2 + \left(\frac{d}{2} - h\right)^2 = \left(\frac{d}{2}\right)^2 \quad \text{(see Fig. 14)}$$

in which b is the diameter of the smallest observable islet area. This area is constant, and could be established by measurement to correspond to an M value (paper value) of 30 mm², which is equivalent to a real area value of 293 μ^2. We then obtain

$$h = \frac{1}{\sqrt{\pi}} \left(\sqrt{m_i} - \sqrt{m_i - 293}\right)$$

By inserting the expressions thus obtained for a, d and h in FLODERUS' reduction formula, we obtain

$$S(n_i) \cdot \frac{5}{5 + \sqrt{\frac{4}{\pi}(m_i - 293)}}$$

which thus gives the number of islets having their centre in the sections examined. If the incidence of islets in the surrounding unexamined 239 sections is assumed to be the same as in the sections counted, the total number of islets in the whole organ, n_c, is obtained through multiplication of this formula by (1 + 239):

$$n_c = S(n_i) \cdot \frac{1{,}200}{5 + \sqrt{\frac{4}{\pi}}\,(m_i - 293)}$$

This can be expressed as a linear function, and depicted graphically as shown in Fig. 15, in which $S(n_i)$ is set off on the abscissa, n_c on the ordinate, and the oblique lines give the different position of the function at varying values of m_i. It is evident that, with this method of calculation, the total number of islets is inversely proportional to the islet size.

An approximate computation of n_c was made from this diagram, and the values obtained are recorded in Tables III: 1 to III: 15. The general order of magnitude of n_c is in fairly good agreement with the data in the literature, calculated in other ways (BARGMANN; BENSLEY; JAFFÉ). This also applies to the considerable individual variation reflected by the n_c values.

Even if n_c reflects, on the whole, the same tendency as the directly counted n_i values, there is reason to question the reliability of the former. The calculations are based on assumptions regarding the shape and distribution of the islets that inevitably imply a considerable approximation (*cf.* p. 79). Moreover, when there is a so markedly asymmetric, positively skew distribution as that represented by the curve for the size of the islets,

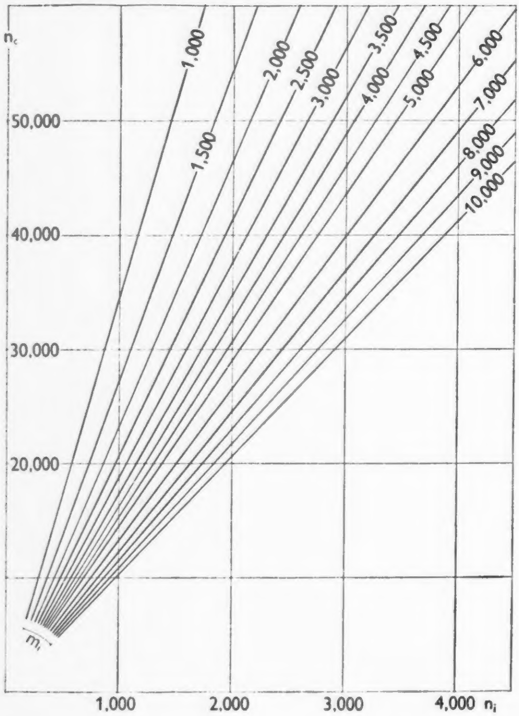

Fig. 15. Diagram for calculation of n_c, when $S(n_i)$ and m_i are known. In this figure, $n_i = S(n_i)$.

FLODERUS' formula gives too low values if a common mean is used for the islets, and the material is not classified according to size. This has been pointed out by BACKMAN, and I was able to confirm it.

Thus, in a constructed example with $S(n_i) = 1{,}700$, I calculated n_c both with a common m_i value, and with the material divided into 10 classes according to size (Table 5). The islet distribution in these classes was varied, so that the computation was made both with a normal distribution, and with varying degrees of asymmetric distribution. It was then found that the difference between the results with the respective methods of calculation becomes greater as the distribution becomes more positively skew: the relatively few large islets are overestimated, and the total number is underestimated when a common m_i value is used.

Finally, in the present material, the inverse proportionality between n_c and m_i expressed by FLODERUS' formula is not, except in group III, in agreement with the directly observed relation between islet number and

TABLE 5

Constructed example of calculation of n_c with FLODERUS' formula, with and without classification according to size

Alternative	$S(n_i)$	With classification acc. to size (10 classes: m_i = 1,000—10,000 μ^2)	Without classification (common m_i)	Difference
I	1,700	23,000	22,000	1,000 = 4.3 %
II	1,700	26,000	23,000	3,000 = 11.5 %
III	1,700	31,000	28,000	3,000 = 9.7 %
IV	1,700	40,000	35,000	5,000 = 12.5 %
V	1,700	43,000	37,000	6,000 = 14.0 %

I = negative asymmetry (many large islets)
II = normal distribution
III = slight positive asymmetry (many small islets)
IV = distinct positive asymmetry (many small islets)
V = marked positive asymmetry (many small islets)

islet size. Here there was, on the contrary, a positive correlation. The same finding was made by BENSLEY in his material. Moreover, on general biologic grounds, such a tendency seems more natural than the reverse.

For the aforementioned reasons, the calculated n_c values are highly approximative and have only been given for their general background value. I did not consider a statistical analysis of them to be warranted.

Alpha and Beta Cell Count

It is an established fact that the proportion of alpha and beta cells normally varies considerably from islet to islet, but that it is relatively constant provided that a sufficiently large number of islet cells are counted (FERNER; HULTQUIST *et al.*). The distribution of the two kinds of cells in the islets, the alpha cells usually lying more peripherally, is probably a contributory cause. For this reason, islets with a small cut surface, which to some extent represent severed peripheral fragments of large islets, contain more alpha cells than islets cut more centrally. On the same grounds, statements of the incidence of islets devoid of alpha cells, as given by JAFFÉ, are of little interest.

Consequently, the number of islets in which the ratio of the two kinds of cells has been determined is of no significance. The chief matter of importance is the total number of cells counted.

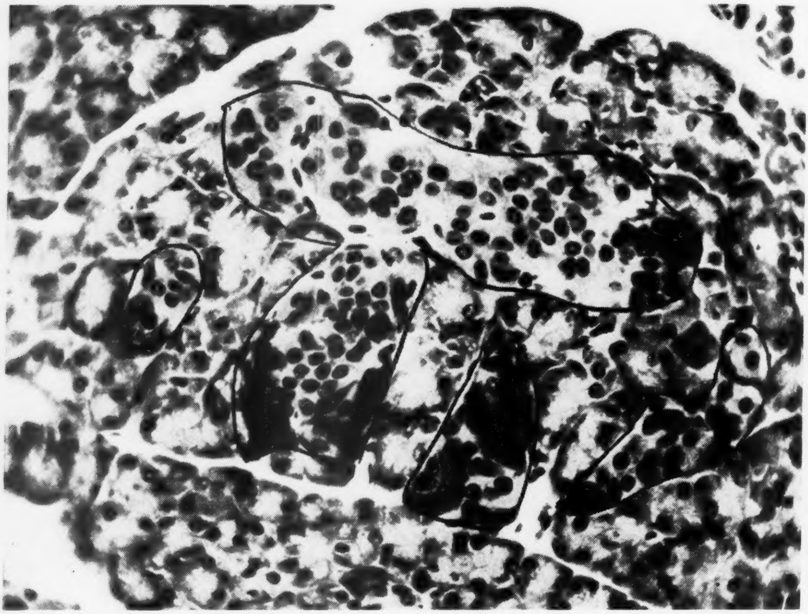

Fig. 16. Pancreas: untreated control (R. 178, group IV A). Four islets in close proximity; the largest consists of two parts, joined only by a relatively narrow area. The normally occurring variation in alpha cell incidence from islet to islet is also seen. (Islet borders reinforced in ink.) Gomori stain. Magnification 300 X.

On the basis of the investigations of HULTQUIST *et al.*, I chose 1,000 cells per animal as a minimum for evaluation of the alpha cell incidence. As may be inferred from Tables IV: 1 to IV: 15, the number ranged from 1,037 to 2,159. The error of the method was computed by comparing the alpha cell incidence obtained in each animal in the D part of the pancreas with that in the L part. For this purpose, the material was assembled in three groups:

1. Non-diabetic groups with functioning exocrine parenchyma
2. Diabetic groups with functioning exocrine parenchyma
3. Groups without functioning exocrine parenchyma (group III).

It was first ascertained in each group whether any systematic difference existed between the D and the L part with respect to the incidence of alpha cells. In the first group, $d = -0.55 \pm 0.79$ per cent; in the second, $d = +1.06 \pm 1.87$ per cent, and in the third group $d = +1.76 \pm 1.45$ per

cent. Thus, no significant difference was present between the two parts in this respect.

The error of a single determination was computed by taking the alpha cell incidence in the two parts of the organ in each animal as a double determination of the same value. The error amounted to $\pm$ 3.87 per cent in the first group, $\pm$5.00 per cent in the second group, $\pm$5.06 per cent in the third group, and $\pm$4.43 per cent in the whole material.

With the staining method used, no differentiation of cell types other than the alpha and beta cells could be made.

General Comments

In drawing the islets for measurement of their size (I), in the islet count (n_i) and in the alpha and beta cell count, the following general principles were applied. Islets of every order of magnitude were counted. Particular attention was focused on the small and smallest islets down to a size of two cells which, as pointed out earlier, corresponded to a surface of 293 μ^2. (*cf.* JAFFÉ's statement that an area of about 300 μ^2 "corresponds to the area of one or two islet cells".) Single cells of an islet cell nature in the parenchyma were not, on the contrary, counted as independent islets. Islets in close proximity were counted as separate islets when they were separated by acinar tissue or (in group III) by connective tissue, but as one islet when they were contiguous (Fig. 16). Such a principle is necessary in order to eliminate subjective criteria to the greatest possible extent, even if it is probable that such a group of islets in close proximity may represent severed extensions of a single large islet.

Finally, it must be stressed that I did not take into account the changes in volume, for example, of the specimen in connexion with fixation and other histologic treatment, since this factor was the same in all the specimens.

CHAPTER 5

BILE FLOW EXPERIMENTS IN NON-DIABETIC ANIMALS

A. BILIARY FISTULA

Biliary fistula experiments were performed on 19 animals (*group I A*). Nine of them (5 males and 4 females) were assigned to category 1, and 10 (3 males and 7 females) to category 2, as follows:

Category 1: R. 101, 103, 106, 108, 110, 122, 129, 134, 136

Category 2: R. 112, 117, 119, 124, 128, 132, 138, 142, 144, 140.

The basis of this division into categories was the length of the post-operative observation period. A complete analysis of the results was not considered warranted unless two, and preferably four, weeks had elapsed since operation. Consequently, the animals which died before this period had elapsed were assigned to category 2.

As described in Chapter 2, *group IV B* served as controls to the biliary fistula animals in category 1. Each of the animals in this group was thus of the same sex and belonged to the same litter as a corresponding animal in group I A. These 9 pairs of animals were pair-fed, *i. e.*, the animal in group IV B was given the same quantity of food as that eaten by the paired animal in group I A.

These 9 pairs consisted of the following animals:

Pair	R. nos.
1	101—102
2	103—104
3	106—107
4	108—109
5	110—111
6	122—123
7	129—133
8	134—135
9	136—137

The course of the experiments and the post mortem findings in these 28 rabbits are summarized in the following animal records.

ANIMAL RECORDS

GROUP I A: CATEGORY 1

R. 101. Male. 1st obs. day 7/1 1953. BW 2.18 kg.—15/1 Glucose tolerance test. BW 2.08 kg. Glucose: 3.4 ml.—20/1 Operation: *Biliary fistula* (glass cannula). BW 2.31 kg. Anaesthetic: 95 mg kemithal.—23/1 Condition good but no bile in bag.—24/1 Bile flow started.—16/2 Glucose tolerance test. BW 2.27 kg. Glucose: 3.3 ml.—19/2 Condition good. Continuous bile flow since 24/1. BW 2.14 kg. Killed. *Autopsy:* moderate reduction in subcutaneous and intra-abdominal adipose tissue. Extensive adhesions around fistula tube. Common bile duct dilated. Liver and pancreas: N.A.D. PW 4.69 g (D 3.02 g, L 1.67 g).—*Micr. exam.:* moderate, perilobular increase in connective tissue of liver.

R. 103. Male. 1st obs. day 20/1 1953. BW 2.50 kg.—22/1 Glucose tolerance test. BW 2.50 kg. Glucose: 4.0 ml.—24/1 Operation: *Biliary fistula* (glass cannula). BW 2.50 kg. Anaesthetic: 100 mg kemithal.—26/1 Condition good. Bile in bag.—16/2 Less bile in bag during past few days.—19/2 Glucose tolerance test. BW 2.55 kg. Glucose: 3.8 ml.—23/2 Condition good. For past few days only 10—20 ml/24 hrs of colourless bile. BW 2.49 kg. Killed. *Autopsy:* moderate reduction in subcutaneous and intra-abdominal adipose tissue. Extensive adhesions around fistula tube and between duodenum and liver. Liver and pancreas: N.A.D. PW 4.90 g (D 2.95 g, L 1.95 g).—*Micr. exam.:* slight increase in connective tissue of liver.

R. 106. Male. 1st obs. day 27/1 1953.—28/1 Glucose tolerance test. BW 2.56 kg. Glucose: 4.1 ml.—30/1 Operation: *Biliary fistula* (polyethylene tubing). BW 2.56 kg. Anaesthetic: 120 mg kemithal.—1/2 Condition good. More than 100 ml/24 hrs of bile in bag.—26/2 Constant copious bile flow. Glucose tolerance test. BW 2.54 kg. Glucose: 4.1 ml.—1/3 Condition good. BW 2.53 kg. Killed. *Autopsy:* moderate reduction in subcutaneous and omental adipose tissue. Disappearance of fatty capsule of kidneys. Extensive loose adhesions between liver, duodenum and colon. Liver and pancreas: N.A.D. PW 3.43 g (D 2.04 g, L 1.39 g).—*Micr. exam.:* some increase in connective tissue of liver.

R. 108. Male. 1st obs. day 3/2 1953. BW 2.81 kg.—6/2 Glucose tolerance test. BW 2.65 kg. Glucose: 4.2 ml.—9/2 Operation: *Biliary fistula* (polyethylene tubing). BW 2.65 kg. Anaesthetic: 75 mg kemithal. When the animal woke after operation, its hind legs were found to be paralyzed.—11/2 Condition fairly good, although paralysis of legs persists. Copious bile flow.—6/3 Paralysis unchanged; fairly good appetite and copious bile flow. Glucose tolerance test unsuccessful.—9/3 Distinct exacerbation during past 2 days. No bile flow since yesterday. Died in morning. *Autopsy* 2 hrs after death: BW 1.52 kg. Almost complete disappearance of subcutaneous and intra-abdominal adipose tissue. Extensive adhesions between liver, stomach and duodenum. Liver: several small white spots close to gallbladder; otherwise N.A.D. Pancreas: N.A.D. PW 3.14 g (D 1.76 g, L 1.38 g). Paralysis probably caused by fracture of a lumbar vertebra during operation.—*Micr. exam.:* Occasional necrotic foci with infiltration of leukocytes in liver.

R. 110. Male. 1st obs. day 17/2 1953.—20/2 Glucose tolerance test. BW 2.72 kg. Glucose: 4.4 ml.—27/2 Operation: *Biliary fistula* (polyethylene tubing). BW 2.78 kg. Anaesthetic: 175 mg kemithal.—1/3 Condition fairly good. Eats and drinks. Copious bile in bag.—19/3 Condition good. During past few days reduction in bile flow from about 60 ml/24 hrs to about 20 ml/24 hrs.—25/3 Glucose tolerance test. BW 2.32 kg. Glucose: 3.7 ml.—28/3 No bile flow past 48 hrs. BW 2.15 kg. Killed. *Autopsy:* considerable general reduction in adipose tissue. Extensive adhesions between liver, duodenum and gallbladder. Greater part of omentum converted into gelatinous mass. Several small white spots on surface and cut surface of liver. Pancreas: N.A.D. PW 5.45 g (D 3.49 g, L 1.96 g).—*Micr. exam.:* signs of intrahepatic purulent cholangitis.

R. 122. Female. 1st obs. day 19/5 1953. BW 1.71 kg.—29/5 Operation: *Biliary fistula* (poly-

ethylene tubing). BW 1.82 kg. Anaesthetic: 145 mg kemithal.—31/5 Condition fairly good. Copious bile flow.—25/6 Glucose tolerance test. BW 1.57 kg. Glucose: 2.3 ml.—28/6 Condition good. Still copious bile flow. BW 1.45 kg. Killed. *Autopsy:* total lack of subcutaneous and intra-abdominal adipose tissue. Moderate adhesions between liver, anterior abdominal wall and duodenum. Liver surface N.A.D. but fairly numerous tough, white streaks of connective tissue on cut surface. Pancreas: N.A.D. PW 3.56 g (D 2.10 g, L 1.46 g).—*Micr. exam.:* increase in perilobular connective tissue of liver.

R. 129. Female. 1st obs. day 2/9 1953. BW 2.99 kg.—4/9 Glucose tolerance test. BW 2.70 kg. Glucose: 4.3 ml.—5/10 Operation: *Biliary fistula* (polyethylene tubing). BW 2.97 kg. Anaesthetic: 300 mg kemithal. Fairly copious bleeding on opening of gallbladder; first bile obtained in bag therefore greatly blood-mixed.—6/10 Bag empty. Since the tubing was suspected to be occluded by a clot, 1 ml of sterile water was injected into it. This was followed shortly by copious flow of clear, reddish-brown fluid, subsequently green.—8/10 Condition good. Copious bile flow.—29/10 Glucose tolerance test. BW 1.95 kg. Glucose: 3.1 ml.—30/10 Definite deterioration; lethargic, no appetite. BW 1.74 kg. Killed. *Autopsy:* complete lack of adipose tissue. Extensive adhesions in superior part of abdomen. Scattered white nodules, size of a pin's head, on both surface and cut surface of liver, which was tough and firm. Bile ducts dilated. In right central lobe, close to diaphragmatic surface, abscess cavity about 20 mm in diameter. Enlargement of mesenteric lymph nodes. Pancreas: N.A.D. PW 3.79 g (D 2.64 g, L 1.15 g).—*Micr. exam.:* necrotic foci and connective tissue increase in liver.

R. 134. Female. 1st obs. day 24/9 1953. BW 3.00 kg.—28/9 Glucose tolerance test. BW 2.90 kg. Glucose: 4.6 ml.—30/9 Operation: *Biliary fistula* (polyethylene tubing). BW 2.89 kg. Anaesthetic: 350 mg kemithal.—2/10 Condition good. Very copious bile in bag.—20/10 Condition strikingly good; appetite excellent. Continuous bile flow, more than 100 ml/24 hrs.—26/10 Glucose tolerance test. BW 2.75 kg. Glucose: 4.4 ml.—1/11 No change in good condition. BW 2.72 kg. Killed. *Autopsy:* fairly great reduction in fat but not total. Remaining adipose tissue lay in thin layer subcutaneously and in posterior abdominal wall; colour yellow, instead of white. Extensive adhesions around fistula tube, and between caudal surface of liver and right abdominal wall. Liver: N.A.D. In pylorus region, pancreas was fairly adherent to liver; it could not be entirely dissected free of connective tissue and vessels. PW 7.44 g (D 4.37 g, L 3.07 g).—*Micr. exam.:* slight perolobular increase in connective tissue of liver.

R. 136. Female. 1st obs. day 25/9 1953. BW 2.65 kg.—1/10 Glucose tolerance test. BW 2.46 kg. Glucose: 3.9 ml.—9/10 Operation: *Biliary fistula* (polyethylene tubing). BW 2.63 kg. Anaesthetic: 350 mg kemithal.—11/10 Condition good. Copious bile flow.—22/10 Still very good condition. Bile flow about 100 ml/24 hrs.—23/10 Acute exacerbation; lethargic, no appetite. Abdomen tense. Died during day. *Autopsy* immediately: BW 2.27 kg. Almost complete lack of adipose tissue. Extensive adhesions around fistula tube, and between duodenum and liver. Cause of death: strangulation ileus, owing to twisting of about 2 dm of jejunum around fistula tube. This part of intestine totally gangrenous. Scattered white spots, size of a pin's head, on surface and cut surface of liver. Pancreas: N.A.D. PW 6.48 g (D 3.29 g, L 3.19 g).—*Micr. exam.:* several small abscess cavities in liver.

GROUP I A: CATEGORY 2

R. 112. Female. 1st obs. day 4/5 1953. BW 2.86 kg.—7/5 Glucose tolerance test. BW 2.73 kg. Glucose: 4.4 ml.—11/5 Operation: *Biliary fistula* (polyethylene tubing). BW 2.67 kg. Anaesthetic: 275 mg kemithal.—13/5 Condition good. Copious bile flow.—19/5 Condition poorer. Appetite decreased. Diminution of bile flow.—21/5 Died during previous night. *Autopsy:* BW 1.78 kg.

Autolysis. No definitely demonstrable cause of death. Liver: N.A.D. PW 3.77 g (D 2.77 g, L 1.00 g).—*Micr. exam.:* no definite pathologic changes in liver.

R. 117. Female. 1st obs. day 15/5 1953. BW 1.86 kg.—18/5 Glucose tolerance test. BW 1.96 kg. Glucose: 3.1 ml.—20/5 Operation: *Biliary fistula* (polyethylene tubing). BW 1.93 kg. Anaesthetic: 160 mg kemithal.—22/5 Condition fairly good. Eats and drinks. Copious bile flow.—25/5 Died during previous night. *Autopsy:* BW 1.44 kg. Autolysis. No definitely demonstrable cause of death. Liver: N.A.D.—No *micr. exam.* made.

R. 119. Male. 1st obs. day 19/5 1953. BW 2.34 kg.—21/5 Glucose tolerance test. BW 2.24 kg. Glucose: 3.3 ml.—23/5 Operation: *Biliary fistula.* BW 2.27 kg. Anaesthetic: 200 mg kemithal. Access to gallbladder difficult, and it burst during introduction of polyethylene tubing; reinforcing sutures could not prevent leakage.—25/5 Died during previous night. *Autopsy:* BW 2.16 kg. Large quantities of bile-stained fluid in abdomen; fibrinous deposits on intestinal serosa. Autolysis.—No *micr. exam.* made.

R. 124. Female. 1st obs. day 29/5 1953. BW 1.70 kg.—1/6 Glucose tolerance test. BW 1.56 kg. Glucose: 2.5 ml.—2/6 Operation: *Biliary fistula* (polyethylene tubing). BW 1.66 kg. Anaesthetic: 125 mg kemithal.—4/6 Condition fairly good. Copious bile flow.—10/6 Condition considerably poorer, lethargic, no appetite; frequent loose defaecations. Died during day. *Autopsy:* BW 1.15 kg. No definitely demonstrable cause of death. Liver: N.A.D. PW 2.26 g (D 1.31 g, L 0.95 g).—*Micr. exam.:* slight perilobular increase in connective tissue of liver.

R. 128. Female. 1st obs. day 2/9 1953. BW 2.97 kg.—4/9 Glucose tolerance test. BW 2.75 kg. Glucose: 4.4 ml.—8/9 Operation: *Biliary fistula* (polyethylene tubing). BW 2.91 kg. Anaesthetic: 225 mg kemithal.— 10/9 Condition somewhat affected. Hind legs paralyzed. Copious bile flow.—12/9 Polyethylene tube had become detached from bag and slid into abdomen. Consequently, no reason to continue experiment; animal killed. BW 2.65 kg. *Autopsy:* no intraperitoneal leakage of bile, but considerable subcutaneous bile infiltration. Liver: N.A.D. PW 4.18 g (D 2.83g, L 1.35 g). Haematoma in psoas muscle possible cause of paralysis.—*Micr. exam.:* liver N.A.D.

R. 132. Female. 1st obs. day 15/9 1953. BW 3.03 kg.—21/9 Glucose tolerance test. BW 2.99 kg. Glucose: 4.8 ml.—24/9 Operation: *Biliary fistula* (polyethylene tubing). BW 2.92 kg. Anaesthetic: 300 mg kemithal. Access to gallbladder difficult.—26/9 Condition fairly good. Copious bile flow.—27/9 Died during previous night. *Autopsy:* BW 2.88 kg. Advanced pregnancy. Autolysis.—No *micr. exam.* made.

R. 138. Female. 1st obs. day 2/10 1953. BW 2.40 kg.—6/10 Glucose tolerance test. BW 2.47 kg. Glucose 4.0 ml.—12/10 Operation: *Biliary fistula* (polyethylene tubing). BW 2.43 kg. Anaesthetic: 250 mg kemithal.—14/10 Condition good. Very copious bile flow.—16/10 Acute exacerbation, lethargic, no appetite. Died in evening. *Autopsy:* BW 1.97 kg. Strangulation ileus; 2—3 dm intestinal loop gangrenous. Liver: N.A.D.—No *micr. exam.* made.

R. 142. Male. 1st obs. day 13/10 1953. BW 2.31 kg.—19/10 Glucose tolerance test. BW 2.01 kg. Glucose: 3.2 ml.—22/10 Operation: *Biliary fistula* (polyethylene tubing introduced into common bile duct with gallbladder intact). BW 1.90 kg. Anaesthetic: 150 mg kemithal.—24/10 Condition fairly good. Scanty bile flow.—31/10 Cessation of bile flow.—2/11 Died during previous night. *Autopsy:* BW 1.70 kg. Considerably dilated common duct, containing soft green concretions, which occluded lumen of tubing. Gallbladder also greatly dilated and ruptured; infiltration of bile into liver parenchyma. Autolysis.—No *micr. exam.* made.

R. 144. Female. 1st obs. day 20/10 1953.—23/10 Glucose tolerance test. BW 2.50 kg. Glucose: 4.0 ml.—27/10 Operation: *Biliary fistula* (polyethylene tubing introduced into common bile duct). BW 2.56 kg. Anaesthetic: 400 mg kemithal.—29/10 Condition fairly good. Bile flow more than 100 ml/24 hrs.—2/11 Condition poorer; lethargic, no appetite. Bile flow unchanged.—4/11 Died during day. *Autopsy:* BW 1.99 kg. Widespread lesions of peritoneum; numerous fibrinous

deposits and loose adhesions between loops of small intestine. Retroperitoneally, an abscess cavity extending from liver to pelvic cavity, filled with black, foetid pus. No *micr. exam.* made.

R. 140. Male. 1st obs. day 13/10 1953.—15/10 Glucose tolerance test. BW 2.41 kg. Glucose: 3.9 ml.—17/10 ***Attempted operation:*** owing to overdosage of anaesthetic, death occurred before opening of abdomen. BW 2.08 kg. ***Autopsy:*** N.A.D. PW 2.94 g (D 1.86 g, L 1.08 g).

GROUP IV B: CATEGORY 1

R. 102. Male. Pair-fed control to R. 101. 1st obs. day 7/1 1953. BW 2.11 kg.—13/1 Glucose tolerance test. BW 1.97 kg. Glucose: 3.2 ml.—20/1 (op. day: R. 101) BW 2.30 kg.—23/2 Glucose tolerance test. BW 2.30 kg. Glucose: 3.7 ml.—26/2 BW 2.30 kg. Killed. ***Autopsy:*** N.A.D. PW 3.70 g (D 1.92 g, L 1.78 g).

R. 104. Male. Pair-fed control to R. 103. 1st obs. day 20/1 1953. BW 2.50 kg.—23/1 Glucose tolerance test. BW 2.33 kg. Glucose: 3.8 ml.—24/1 (op. day: R. 103) BW 2.34 kg.—3/3 Glucose tolerance test. BW 2.24 kg. Glucose: 3.6 ml.—5/3 BW 2.28 kg. Killed. ***Autopsy:*** N.A.D. PW 2.78 g (D 1.81 g, L 0.97 g).

R. 107. Male. Pair-fed control to R. 106. 1st obs. day 27/1 1953. BW 2.78 kg.—29/1 Glucose tolerance test. BW 2.55 kg. Glucose: 4.1 ml.—30/1 (op. day: R. 106) BW 2.69 kg.—26/2 Glucose tolerance test. BW 2.61 kg. Glucose: 4.2 ml.—2/3 BW 2.88 kg. Killed. ***Autopsy:*** N.A.D. PW 3.82 g (D 2.33 g, L 1.49 g).

R. 109. Male. Pair-fed control to R. 108. 1st obs. day 3/2 1953. BW 2.88 kg.—8/2 Glucose tolerance test. BW 2.67 kg. Glucose: 4.3 ml.—9/2 (op. day: R. 108) BW 2.71 kg.—9/3 Glucose tolerance test. BW 2.60 kg. Glucose: 4.2 ml.—12/3 BW 2.63 kg. Killed. ***Autopsy:*** N.A.D. PW 3.42 g (D 1.86 g, L 1.56 g).

R. 111. Male. Pair-fed control to R. 110. 1st obs. day 17/2 1953. BW 2.51 kg.—25/2 Glucose tolerance test. BW 2.48 kg. Glucose: 4.0 ml.—27/2 (op. day: R. 110) BW 2.49 kg.—27/3 Glucose tolerance test. BW 2.67 kg. Glucose: 4.3 ml.—29/3 BW 2.54 kg. Killed. ***Autopsy:*** N.A.D. PW 2.94 g (D 1.50 g, L 1.44 g).

R. 123. Female. Pair-fed control to R. 122. 1st obs. day 19/5 1953. BW 1.55 kg.—29/5 (op. day: R. 122) BW 1.68 kg.—25/6 Glucose tolerance test. BW 2.03 kg. Glucose: 3.3 ml.—29/6 BW 1.98 kg. Killed. ***Autopsy:*** N.A.D. PW 3.69 g (D 2.70 g, L 0.99 g).

R. 133. Female. Pair-fed control to R. 129. 1st obs. day 15/9 1953. BW 2.33 kg.—25/9 Glucose tolerance test. BW 2.55 kg. Glucose: 4.1 ml.—5/10 (op. day: R. 129) BW 2.61 kg.—29/10 Glucose tolerance test. BW 2.74 kg. Glucose: 4.4 ml.—3/11 BW 2.59 kg. Killed. ***Autopsy:*** N.A.D. PW 3.80 g (D 2.39 g, L 1.41 g).

R. 135. Female. Pair-fed control to R. 134. 1st obs. day 24/9 1953. BW 2.83 kg.—28/9 Glucose tolerance test. BW 2.64 kg. Glucose: 4.9 ml.—30/9 (op. day: R. 134) BW 2.66 kg.—26/10 Glucose tolerance test. BW 2.98 kg. Glucose: 4.8 ml.—3/11 BW 2.98 kg. Killed. ***Autopsy:*** N.A.D. PW 3.36 g (D 2.14 g, L 1.22 g).

R. 137. Female. Pair-fed control to R. 136. 1st obs. day 25/9 1953. BW 2.27 kg.—1/10 Glucose tolerance test. BW 2.28 kg. Glucose: 3.7 ml.—9/10 (op. day: R. 136) BW 2.30 kg.—26/10 Glucose tolerance test. BW 2.34 kg. Glucose: 3.7 ml.—28/10 BW 2.38 kg. Killed. ***Autopsy:*** N.A.D. PW 4.83 g (D 3.60 g, L 1.23 g).

POSTOPERATIVE COURSE

Observation Period

The mean postoperative observation period in the biliary fistula animals in category 1 was 27.6 days, the figures for the individual animals being 30, 30, 30, 28, 29, 30, 25, 32 and 14 days. The corresponding mean figure for the animals in category 2 was 5.5 days, and the individual duration 10, 5, 2, 8, 4, 3, 4, 11, 8 and 0 days. In group IV B the corresponding time, *i.e.*, from the operation day of the paired animal to the death of the control, was a mean 31.3 days. For various reasons, it was not possible to kill the control and the experimental animal on the same day; this explains why the observation period was slightly longer in the former animals.

General Condition

The general condition of the operated animals in category 1 was slightly affected in that their appetite was impaired to a varying extent. Otherwise, they were lively and unaffected, with two exceptions: R. 108 and R. 129. In the former, paralysis of the hind legs appeared immediately after operation, presumably due to fracture of a vertebra. Despite this, the animal was relatively unaffected, and the bile flow was copious during the first three weeks. Thereafter, the condition deteriorated successively and death occurred 28 days after operation. Autopsy was performed immediately after death, and showed occasional circumscribed necroses of the liver. R. 129 was also in fairly good condition during the first three weeks after operation, but subsequently became worse and was killed on the 25th day when moribund. In this case as well, small necrotic foci were present in the liver, in addition to a somewhat larger subdiaphragmatic abscess.

R. 136 died 14 days after operation of acute strangulation ileus, but its condition was good until the day of death; autopsy was performed immediately afterwards. None of the other animals in this group died spontaneously, but all were killed at the end of the experimental period.

In the animals in category 2, the cause of premature death was as follows: in 3 cases (R. 119, 142 and 144) rupture of sutures with leakage of bile into the abdominal cavity and peritonitis, in 1 (R. 124) signs of enterocolitis with diarrhoea, in 1 (R. 128) detachment of the fistula tubing, in 1 (R. 138) strangulation ileus, and in 1 (R. 140) overdosage of anaesthetic. In 3 cases (R. 112, 117 and 132) the cause of death could not be established, although one of these animals (R. 132) was found at autopsy to be pregnant.

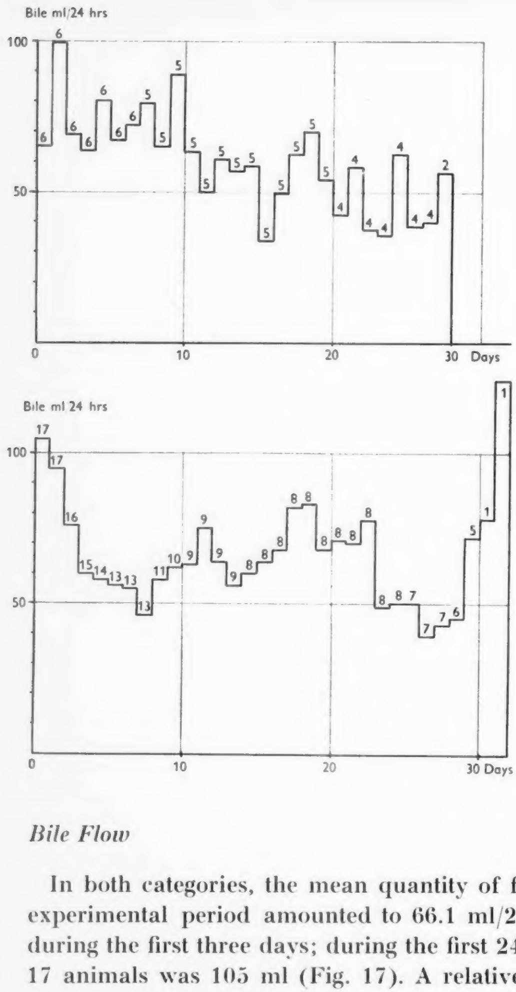

Fig. 17. Bile flow in groups I A (lower diagram) and I B (upper diagram): mean values. Figure above each column denotes number of observations. Mean value for whole experimental period: group I A: 66.1 ml/24 hrs; group I B: 61.7 ml/24 hrs.

Bile Flow

In both categories, the mean quantity of fistular bile during the whole experimental period amounted to 66.1 ml/24 hrs. The flow was greatest during the first three days; during the first 24 hours, the mean quantity in 17 animals was 105 ml (Fig. 17). A relatively large individual variation was noted. For example, during practically the whole 32-day observation period, a flow of between 100 and 150 ml/24 hrs was recorded in R. 134, whereas the corresponding figure in other animals was 20 to 30 ml. The highest 24-hour output was noted in R. 128; during the first 24 hours after operation it amounted to 227 ml. In two animals in category 1 (R. 108 and 110) the bile flow ceased entirely during the last and the last two days, respectively, of the observation period. At autopsy, the polyethylene tubing was found to be occluded by concretions.

TABLE 6

Electrolyte and glucose composition of fistula bile in groups I A and I B
(34 determinations in 18 animals in group I A and 16 determinations in 5 animals in group I B)

Electrolyte mEq./Lit.	Group	n	$\bar{x} \pm e(\bar{x})$	s	Differences	t	P $\sim$
Na^+	I A	18	151.8 ± 2.23	9.45	11.2 ± 4.68	2.39	0.02*
	I B	5	140.6 ± 3.76	8.41			
K^+	I A	18	4.80 ± 0.17	0.72	0.58 ± 0.35	1.65	0.1
	I B	5	5.38 ± 0.26	0.58			
Cl^-	I A	18	88.4 ± 2.72	11.53	6.8 ± 5.61	1.21	0.2
	I B	5	81.6 ± 4.06	9.07			
HCO_3^-	I A	18	46.2 ± 2.72	11.56	25.4 ± 6.22	4.09	< 0.001***
	I B	5	20.8 ± 6.72	15.02			
Glucose mg/100 ml	I B	5	61.6 ± 16.02	35.82	—	—	—

During the first few days after operation the bile was thin, pale green and transparent; it subsequently became more concentrated, dark green and opaque, although no concretions were visible macroscopically. In three cases (R. 103, 110 and 122) the bile once more became lighter, yellowish-green and thinner towards the end of the experimental period.

The *electrolyte content* of the fistular bile was determined on 34 occasions in 18 animals. The results are recorded in Table 6. The values obtained are largely in agreement with those found by earlier workers (Cooke & Crowley; Gamble; Ravdin *et al.*) and with the composition of the replacement fluid administered (*cf.* p. 46).

Body Weight

A comparison between the postoperative changes in body weight (BW) in operated animals (category 1) and in the controls is shown in Fig. 18. At the beginning of the experimental period, the BW was somewhat higher in the former than in the corresponding paired animals; at the end of the period the reverse applied. Although both groups were given the same amount of food, the operated animals showed a mean weight loss of 18.0 g/24 hrs, whereas a mean gain of 2.9 g/24 hrs was recorded in the controls. In two animals (R. 108 and 129) the weight loss was considerably greater than in the others. These were the two animals in which the con-

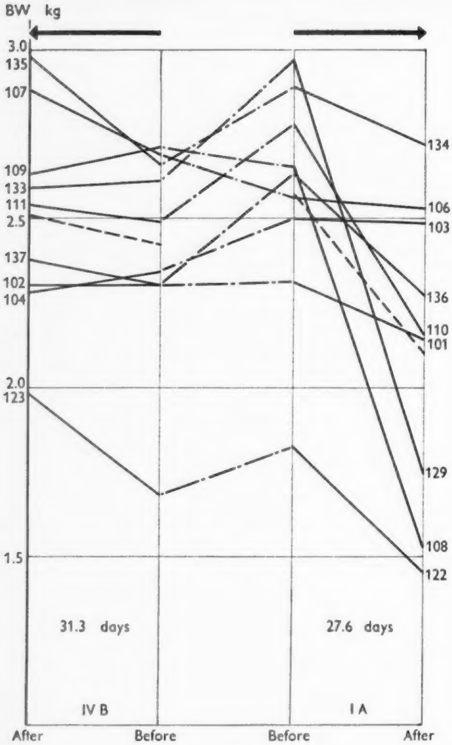

Fig. 18. Change in BW during postoperative observation period: group I A (biliary fistula) and group IV B (controls).
— — — — — mean value
—·—·—·— paired animals.
Before = before operation.
After = after operation.

dition was poor during the last few days of life, and degenerative changes in the liver were found at autopsy.

GLUCOSE TOLERANCE TESTS

Glucose tolerance tests were made in the pair-fed groups before operation of the animals in group I A (at a corresponding time in the controls), as well as a mean 26.1 days (group I A) and 27.7 days (group IV B) after this operation. No preoperative test was, however, made in pair 6. The test could not be done postoperatively in R. 108 owing to its poor condition, nor in R. 136 on account of its sudden and unexpected death. The results of the individual tests are recorded in Tables I: 1—I: 4, and in Figs. 19—22.

TABLE 7

Comparison of glucose tolerance areas of paired animals in groups I A and IV B before and after operation of animals in group I A (*cf.* Figs. 19—22)
(The sign — before a difference denotes a greater tolerance area = lower glucose tolerance in group I A than in group IV B, the sign + the contrary)

Pair no.	Animals nos.	Tolerance area (mg-min)						
		Before operation			After operation			d_1—d_2
		Groups		Difference	Groups		Difference	
		I A	IV B	d_1	I A	IV B	d_2	
1	R. 101—102	4,485	3,918	— 567	2,285	6,680	+ 4,395	4,962
2	R. 103—104	3,168	1,803	— 1,365	3,335	3,580	+ 245	1,610
3	R. 106—107	3,960	3,303	— 657	2,780	5,055	+ 2,275	2,932
4	R. 108—109	4,858	3,078	— 1,780	—	5,375	—	—
5	R. 110—111	4,710	5,560	+ 850	2,425	6,910	+ 4,485	3,635
6	R. 122—123	—	—	—	1,090	2,435	+ 1,345	—
7	R. 129—133	6,990	6,080	— 910	10,275	5,485	— 4,790	3,880
8	R. 134—135	6,950	7,335	+ 385	6,025	7,235	+ 1,210	825
9	R. 136—137	6,910	6,745	— 165	—	5,920	—	—
Test of differences					alt. a		alt. b	
	d		— 526.1		+ 1,309.3		+ 2,325.8	2,974
	s_d		869.8		3,133		1,760	1,529
	$e(\bar{d})$		307.6		1,184		719	624
	t		1.710		1.106		3.236	4.764
	P		0.1		0.3		0.02	0.01

It is evident that, both before and after operation, the glucose tolerance curves were entirely within the normal range of variation, and the tests of deviation from the "normal group" (*cf.* p. 42) between the mean values of the tolerance areas showed no significant difference (Table 26). A comparison between the two groups nevertheless showed a tendency to a slightly lower tolerance in group I A before operation, but the difference is not significant ($P \sim 0.1$): Table 7. After operation, the reverse applied, *i. e.*, a tendency to a slightly higher tolerance in group I A, although the difference is still not significant ($P \sim 0.3$): Table 7, alt. a.

As may be inferred from Fig. 21 and Table I:3, the postoperative curves in group I A showed a high tolerance with only two exceptions: R. 129, in which the curve was of a distinctly "diabetic" nature, and R. 134. R. 129 was the animal in which degenerative changes were present in the liver. If, in view of this fact, the animal is excluded from the statistical analysis (Table 7, alt. b), a probably significant difference is present between the two groups ($P \sim 0.02$). Finally, if the difference between the differences,

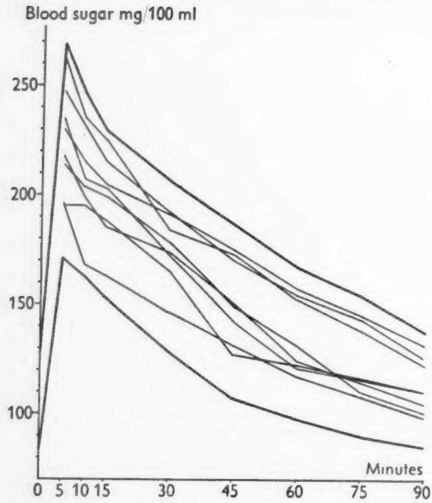

Fig. 19. Glucose tolerance curves: group I A before operation.

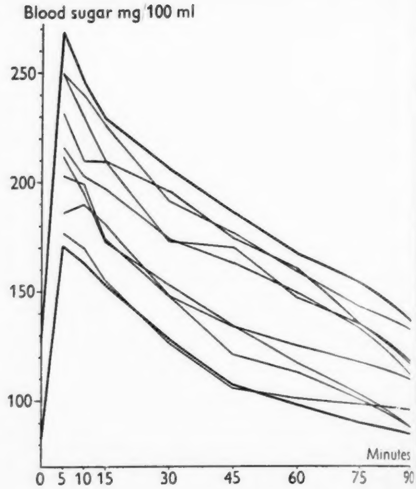

Fig. 20. Glucose tolerance curves: group IV B before operation of paired animals in group I A.

In these and all similar figures, the thick lines denote the ± 2 s limits of variation (*cf.* Fig. 2).

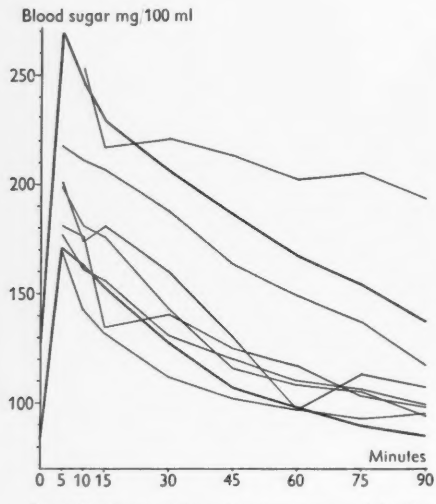

Fig. 21. Glucose tolerance curves: group I A after operation.

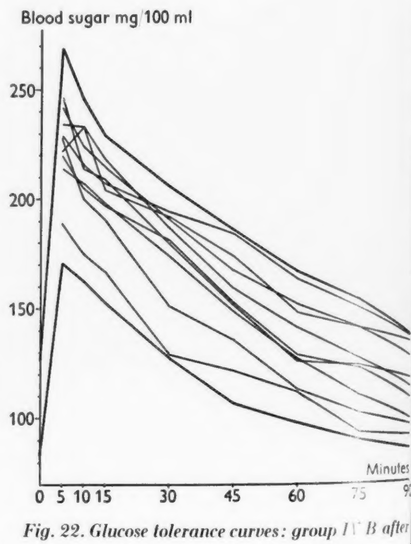

Fig. 22. Glucose tolerance curves: group IV B after operation of paired animals in group I A.

$d_1 - d_2$, is analyzed, a significant difference is also obtained ($P \sim 0.01$). This lends further support to the view that a change in glucose tolerance had taken place in the two paired groups of animals.

AUTOPSY

General Observations

A more or less marked reduction in the macroscopically visible *adipose tissue* subcutaneously, in the omentum and the posterior abdominal wall was observed in all the biliary fistula animals in category 1. In 4 cases practically no adipose tissue was present; in the other 5 the reduction was considerable, although not complete. In such cases, the remaining adipose tissue was of a flabby, "myxoedematous" nature. Microscopic examination showed that the fat droplets, which normally fill the entire cell, had decreased considerably in size or disappeared, so that the tissue had assumed

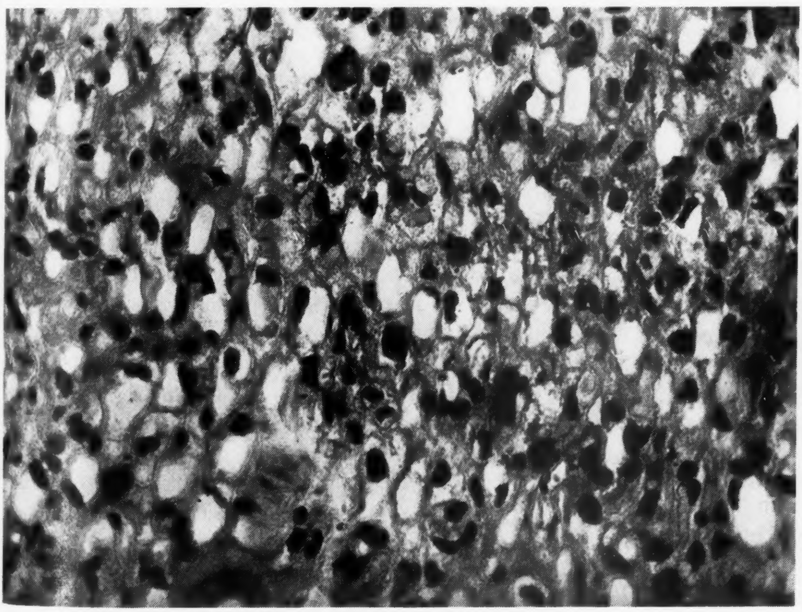

Fig. 23. Perirenal adipose tissue (R. 149, group II B). Shows the typical picture of adipose tissue atrophy observed in bile flow experiments. Haematoxylin and eosin stain. Magnification 490 X. Note similarity to serous adipose tissue atrophy in man.

an undifferentiated, mesenchymal nature (Fig. 23). The loss of adipose tissue was considerable even in animals that had not exhibited any great weight loss during the experimental period. In the controls, the adipose tissue had the normal appearance throughout.

Jaundice was not observed at autopsy in any of the biliary fistula animals. In 4 cases (R. 108, 110, 129 and 136), signs of purulent cholangitis were visible macroscopically.

Pancreas Weight

Although the interstitial adipose tissue of the pancreas was considerably reduced, the total pancreas weight (PW), *i. e.*, parenchyma plus interstitial tissue, was consistently greater in the biliary fistula animals than in the controls (Table 10). The difference is probably significant ($P \sim 0.05$). If the pancreas weight is expressed in g/kg of body weight (PW/BW), the difference is highly significant ($P \sim 0.001$). The degree of significance was the same irrespective of whether the comparison was made between the PW and the PW/BW, respectively, in the individual pairs of animals (Table 8), or between the means for each of the groups.

QUANTITATIVE MICROMORPHOLOGIC ANALYSES

Size of Total Pancreas Parenchyma

The size of the total pancreas parenchyma (V_p) was also larger in the operated animals. Thus, the mean value in group I A was 1,394.7 mm³ and that in group IV B was 978.9 mm³; the difference, 415.8 ±156.1 mm³, being probably significiant ($P \sim 0.02$). Expressed as V_p/BW, the mean value was 671.6 mm³ in group I A and 396.8 mm³ in group IV B. The difference, 274.8 ±69.6 mm³, is highly significant ($P \sim 0.001$). Comparison between the paired animals in the respective groups also showed highly significant differences for both V_p and V_p/BW (Table 8).

Size of Islet Tissue

The results of the quantitative analysis of the islet tissue in the two paired groups of animals are recorded in Table 11. Only 8 pairs could be compared, since in pair 5 (R. 110 and 111) staining was unsuccessful, so that satisfactory identification of the islets was impossible. Consequently, only the total parenchymal volume could be determined in this pair.

TABLE 8

Analysis of differences between paired animals in groups I A and IV B
(The sign + before a difference denotes a lower value in group I A than in group IV B, the sign — the contrary)

Measured quantity	$\bar{d}$	s_d	$e(\bar{d})$	t	P ∾
BW kg					
Before op.	— 0.148	0.175	0.058	2.535	0.05*
After op.	+ 0.394	0.397	0.132	2.983	0.02*
BS dm² (After op.)	+ 1.244	1.333	0.444	2.802	0.02*
PW g	— 1.167	1.544	0.515	2.267	0.05*
PW/*BW* g	— 0.803	0.457	0.152	5.274	0.001***
V_p mm³	— 415.8	150.4	50.13	8.294	0.001***
V_p/BW mm³	— 274.8	141.6	47.20	5.822	0.001***
n_i	— 503.1	392.0	138.6	3.630	0.01**
n_i/BW	— 366.5	156.0	55.16	6.644	0.001***
m_i μ^2	— 441.8	306.6	108.4	4.076	0.01**
V_i mm³	— 2.846	1.920	0.679	4.192	0.01**
V_i/BW mm³	— 1.774	0.415	0.147	12.083	0.001***
α %	+ 8.03	6.46	2.29	3.515	0.01**

In the remaining 8 pairs, the sample of islets taken for measurement of the average islet size was larger than in the rest of the material, the mean value of N being 601 in group I A, and 594 in group IV B. The reason is that, as discussed in Chapter 4 (p. 81), these two groups were used for the preliminary investigation of the islet size, for calculation of the size of the sample necessary for this determination. Since the 500 islets per animal used for this investigation were taken from only 4 sections in each animal, the sample was later supplemented by about 100 islets from remaining sections.

The size of the islet tissue was considerably larger in the operated group than in the control group. This was as distinct in analyses of the differences between the paired animals (Table 8) as in comparisons between the mean values for the whole groups. The difference appeared both in the number of islets (n_i) and in their size (m_i) and, consequently, in the islet volume (V_i) as well. As may be inferred from Table 8, comparison

between the paired animals showed significant or highly significant differences in every respect. In comparisons between the mean values for the groups, the differences were highly significant with respect to n_i, n_i/BW, V_i and V_i/BW, but only probably significant for m_i. The difference was most conspicuous in $\bar{d}$ for V_i/BW, the t value being 12.083.

Alpha and Beta Cell Count

The alpha and beta cell count showed a lower incidence of alpha cells in group I A than in group IV B. The mean value was 13.51 per cent, whereas in group IV B it was 21.34 per cent (Tables IV: 1 and IV: 2, Appendix). The difference between these means was significant ($P \sim 0.01$), as was also the case in an analysis of the differences between the paired animals. In one animal (R. 136) the alpha cell incidence was extremely low, *i. e.*, 6.34 per cent. This was the animal with the shortest postoperative observation period (14 days). There was no reason to believe that technical factors were responsible for this low value, since the Gomori staining was as distinct in this case as in the others.

HISTOLOGIC AND HISTOCHEMICAL EXAMINATION

Pancreas

The *general appearance* of the pancreatic tissue in the biliary fistula animals exhibited no essentially abnormal features. The reduction in inter- and intralobular adipose tissue was, however, striking in most cases. No qualitative changes were observed in the acinar parenchyma, the vessels or the ducts. In the *islet tissue*, high vascularity and nuclear polymorphism of the beta cells was noted, in addition to the quantitative changes already described. No detailed cytologic studies were, however, made. Fig. 24 shows an example of a fairly typical, large islet from a biliary fistula animal, killed 30 days after operation.

Other Organs

The *lipid content* of the *liver cells* was low in the biliary fistula animals as compared to the controls. The semi-quantitative grading in the individual animals was as follows: group I A: 0, 2, 0, 0, 0, 0, 0, 4, 0; group IV B: 5, 2, 2, 5, 5, 4, 5, 6, 5. The average was thus 0—1 in the former group and 4—5 in the latter (Table. 12).

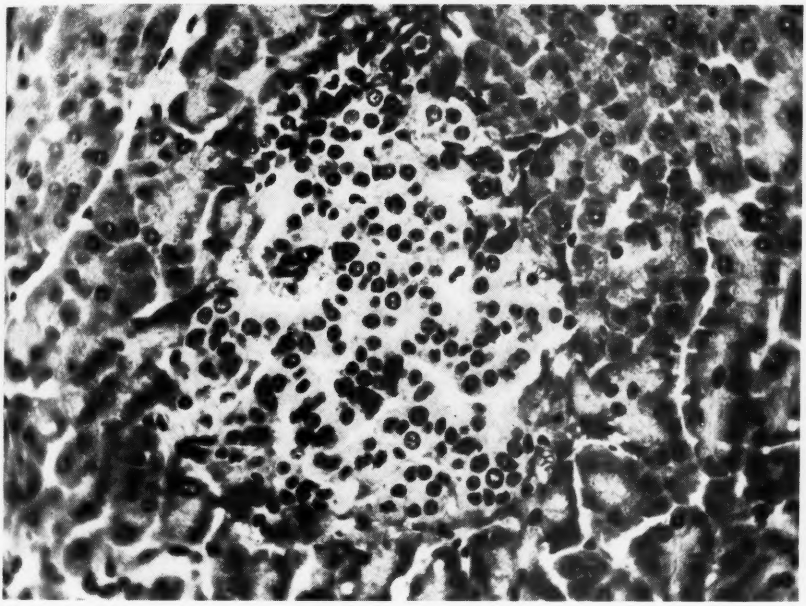

Fig. 24. Pancreas: biliary fistula animal (R. 106, group I A). Example of fairly large islet with low alpha cell incidence, high vascularity and nuclear polymorphism. Gomori stain. Magnification 300 X.

The corresponding figures for the *glycogen content* of the *liver cells* were: group I A: 6, 1, 4, 5, 5, 1, 4 (no determination made in R. 108 and 136); group IV B: 0, 0, 5, 7, 5, 6, 4, 5, 2. This gives an average of 3—4 in both groups, and thus no difference between them in this respect.

Finally, the following grading was made of the *alkaline phosphatase activity* in the *liver cells*. Group I A: 2, 6, 6, 0, 0, 1, 6, 6, 4; group IV B: 5, 6, 4, 6, 6, 6, 6, 6, 6, *i. e.*, an average of 3—4 in the former group and 5—6 in the latter. Thus, here as well, no marked difference was found between the groups. The phosphatase activity was observed at the typical sites in the cell membranes and the bile capillaries. The two cases in group I A in which no phosphatase activity was found were R. 108 and R. 110. These animals had been found at autopsy to exhibit signs of cholangitis, with necrotic foci in the liver. Although circumscribed necrotic areas were also present in the liver of R. 129 and R. 136, a moderate degree of phosphatase activity was demonstrated. In the other 5 animals, microscopic

examination disclosed no signs of infection of the liver, although slight fibrosis was a regular feature.

The *phosphatase content* of the *duodenal mucosa* was high in both groups. The lipid content of the cells of the *adrenal cortex* was lower in all the biliary fistula animals than in the corresponding controls.

B. LIGATION OF COMMON BILE DUCT

Ligation of the common bile duct was performed in 22 animals (*group II A*). Eleven of them (6 males and 5 females) were assigned to category 1 and 11 (5 males and 6 females) to category 2, as follows:

Category 1: R. 113, 114, 116, 125, 126, 156, 160, 166, 213, 214, 219.

Category 2: R. 158, 159, 162, 220, 221, 222, 223, 224, 233, 234, 163.

As in the biliary fistula experiments, assignation to the respective categories was determined by the length of the postoperative observation period. A complete micromorphologic analysis was regarded as uncalled for unless this period amounted to between two and three weeks. One animal, R. 219, was nevertheless assigned to category 1 although only 12 days had elapsed since operation. It is true that in a few cases in category 2 the observation period was more than two weeks, but these animals exhibited post mortem changes that prevented microscopic examination. In one additional case in this category (R. 163), the postoperative observation period was more than three weeks, but it was found at autopsy that recanalization had taken place between the common duct and the duodenum. Consequently, microscopic examination was of no interest.

As I stated in Chapter 2, it proved impossible to obtain a complete control group of paired animals to those with obstruction of the common duct, in the same way as in the biliary fistula animals. The reason was the relatively high mortality during the first two weeks after operation. The four animals in *group IV C* (2 males and 2 females) nevertheless comprised pair-fed controls to four animals in group II A, as follows:

Pair	R. nos.
1	114—115
2	126—127
3	156—157
4	160—161

I considered that group IV C did not suffice as a control group to the whole operated group. Furthermore, the condition and appetite of the operated animals in these four pairs were better than in the majority of the other operated animals, in which the degree of undernutrition was more conspicuous. For these reasons, the controls to group II A were supplemented by an independent group, *group IV E* (2 males and 2 females). During a certain period, these animals were underfed to about the same extent as the operated animals in group II A. The animals in group IV E consisted of the following: R. 169, 170, 171 and 172.

In the statistical analyses of the differences between group II A and the two control groups, no comparisons were made by pair as in groups I A and IV B, owing to the small number of complete pairs. The comparisons were confined to the differences between the mean values in the respective groups.

The main data regarding the experimental course and the post mortem findings in these 30 experiments are given in the following animal records.

ANIMAL RECORDS

GROUP II A: CATEGORY 1

R. 113. Female. 1st obs. day 4/5 1953. BW 2.37 kg.—7/5 Glucose tolerance test. BW 2.32 kg. Glucose: 3.7 ml.—26/5 Glucose tolerance test. BW 2.47 kg. Glucose: 3.9 ml.—28/5 to 26/6 *Period of undernutrition.*—26/6 Glucose tolerance test. BW 2.27 kg. Glucose: 3.6 ml. Undernutrition discontinued; subsequently normal diet.—20/8 Operation: *Ligation of common bile duct.* BW 3.07 kg. Anaesthetic: 300 mg kemithal.—24/8 Condition good. Appetite normal.—9/9 Condition and appetite still unaffected, despite visible jaundice.—10/9 Glucose tolerance test. BW 2.63 kg. Glucose: 4.2 ml.—16/9 Condition considerably poorer; lethargic, no appetite. BW 2.51 kg. Killed. *Autopsy:* generalized jaundice. Total lack of adipose tissue. Large quantity of clear yellow fluid in abdominal cavity. Bile ducts greatly dilated and walls thickened. Diameter of gallbladder about 5 cm; contained dark green bile under strong pressure. Liver brownish-yellow, firm, tough. Slight splenomegaly. Other organs : N.A.D. PW 6.23 g (D 5.19 g, L 1.04 g).—*Micr. exam.:* Liver, biliary cirrhosis; large-dropped fatty degeneration of cirrhotic connective tissue. No fat in liver cells.

R. 114. Female. 1st obs. day 5/5 1953. BW 1.89 kg.—13/5 Glucose tolerance test. BW 1.84 kg. Glucose: 3.0 ml.—15/5 Operation: *Ligation of common bile duct.* BW 1.89 kg. Anaesthetic: 140 mg kemithal. The intention was to create a biliary fistula. After ligation and division of common duct, access to gallbladder proved difficult and it was partly intrahepatic. Attempt to introduce catheter was unsuccessful. Leakage could, however, be prevented by pulling together tobacco bag suture in gallbladder.—17/5 Condition good.—24/5 Distinct jaundice.—26/5 Glucose tolerance test. BW 1.63 kg. Glucose: 2.6 ml.—10/6 Found dead in morning. Except for jaundice, no signs of illness on previous day. *Autopsy:* BW 1.63 kg. Almost total lack of adipose tissue. Small quantity of clear yellow fluid in abdominal cavity. Liver considerably enlarged, tough and firm. Bile ducts greatly dilated, contained clear yellow bile. Moderate dilatation of gallbladder. Other organs: N.A.D. Inappreciable post mortem changes. PW 5.81 g (D 3.54 g, L 2.27 g).—*Micr.*

exam.: liver, marked biliary cirrhosis; large-dropped fatty degeneration of cirrhotic connective tissue. No fat in liver cells.

R. 116. Female. 1st obs. day 15/5 1953. BW 1.88 kg.—18/5 Glucose tolerance test. BW 1.81 kg. Glucose: 2.9 ml.—28/5 to 26/6 *Period of undernutrition.*—26/6 Glucose tolerance test. BW 1.86 kg. Glucose: 3.0 ml. Undernutrition discontinued; subsequently normal diet.—29/8 Operation: *Ligation of common bile duct.* BW 2.78 kg. Anaesthetic: 250 mg kemithal.—31/8 Condition good, appetite normal.—21/9 Glucose tolerance test. BW 2.51 kg. Glucose: 4.0 ml.—25/9 Condition and appetite still unaffected. BW 2.41 kg. Killed. *Autopsy:* moderate generalized jaundice. Small quantity of clear yellow fluid in abdominal cavity. Considerable but not total reduction in adipose tissue. Bile ducts greatly dilated. Liver pale brown, firm and tough. Other organs N.A.D. PW 10.86 g (D 8.11 g, L 2.75 g).—*Micr. exam.:* liver, biliary cirrhosis; moderate fatty degeneration of cirrhotic connective tissue. No fat in liver cells.

R. 125. Female. 1st obs. day 29/5 1953. BW 1.64 kg.—1/6 Glucose tolerance test. BW 1.61 kg. Glucose: 2.6 ml.—28/8 Glucose tolerance test. BW 3.09 kg. Glucose: 4.9 ml.—31/8 Operation: *Ligation of common bile duct.* BW 2.93 kg. Anaesthetic: 250 mg kemithal.—2/9 Condition and appetite good.—22/9 For past few days, lethargic, appetite poor. Today, moribund. Died during day. *Autopsy* immediately after death: BW 2.80 kg. Generalized jaundice. Total lack of adipose tissue. About 500 ml of yellowish-brown, turbid fluid in abdominal cavity. Fibrinous deposits on intestines. Bile ducts dilated. Liver firm, tough. No post mortem changes. Otherwise N.A.D. PW 6.73 g (D 5.00 g, L 1.73 g).—*Micr. exam.:* liver, biliary cirrhosis.

R. 126. Female. 1st obs. day 29/5 1953. BW 1.52 kg.—5/6 Glucose tolerance test. BW 1.59 kg. Glucose: 2.5 ml.—2/9 Operation: *Ligation of common bile duct.* BW 3.07 kg. Anaesthetic: 225 mg kemithal.—4/9 Condition and appetite good.—22/9 Found dead in morning. No noteworthy signs of illness on previous day. *Autopsy:* BW 2.84 kg. Generalized jaundice. Total lack of adipose tissue. About 300 ml of yellow turbid fluid in abdominal cavity. Bile ducts greatly dilated. Liver firm and tough. Otherwise N.A.D. PW 6.76 g (D 4.54 g, L 2.22 g).—*Micr. exam.:* liver, biliary cirrhosis; moderate fatty degeneration of cirrhotic connective tissue. No fat in liver cells.

R. 156. Male. 1st obs. day 13/1 1954. BW 2.19 kg.—20/1 Operation: *Ligation of common bile duct.* BW 2.18 kg. Anaesthetic: ether.—22/1 Condition fairly good; eats.—4/2 Glucose tolerance test. BW 2.32 kg. Glucose: 3.7 ml.—9/2 Condition and appetite good. BW 2.48 kg. Killed. *Autopsy:* generalized jaundice. No noteworthy reduction in fat, but yellow throughout. Bile ducts dilated. Liver somewhat firmer than normally. Otherwise N.A.D. PW 5.78 g (D 3.75 g, L 2.03 g).—*Micr. exam.:* liver, biliary cirrhosis.

R. 160. Male. 1st obs. day 20/1 1954. BW 2.16 kg.—3/2 Operation: *Ligation of common bile duct.* BW 2.30 kg. Anaesthetic: ether.—5/2 Condition and appetite good.—23/2 Glucose tolerance test. BW 2.37 kg. Glucose: 3.8 ml.—26/2 Condition poorer; lethargic, appetite poor. Moribund in evening; killed. BW 2.45 kg. *Autopsy:* Generalized jaundice. Almost total lack of adipose tissue. About 500 ml of green turbid fluid in abdominal cavity, part of which was encapsulated in a sac of omentum. Bile ducts moderately dilated. Liver firm and tough. Pancreas covered with green fibrin; could not therefore be entirely dissected free from surrounding tissues. PW 9.09 g (D 5.45 g, L 3.64 g).—*Micr. exam.:* moderate perilobular increase in connective tissue of liver.

R. 166. Male. 1st obs. day 2/2 1954. BW 2.41 kg.—9/2 Operation: *Ligation of common bile duct.* BW 2.29 kg. Anaesthetic: ether.—11/2 Condition fairly good. Eats.—2/3 Glucose tolerance test. BW 2.33 kg. Glucose: 3.7 ml.—15/3 Condition and appetite still good, despite visible jaundice. BW 2.42 kg. Killed. *Autopsy:* generalized jaundice. Total lack of adipose tissue. Bile ducts dilated; contained clear colourless fluid. Liver enlarged, firm and tough. Otherwise N.A.D. PW 7.60 g (D 4.34 g, L 3.26 g).—*Micr. exam.:* liver, marked biliary cirrhosis; small quantity of fat droplets in connective tissue. No fat in liver cells.

R. 213. Male. 1st obs. day 10/8 1954. BW 2.42 kg.—12/8 Operation: *Ligation of common bile duct.* BW 2.44 kg. Anaesthetic: ether.—14/8 Condition fairly good.—23/8 Somewhat lethargic; distinct jaundice, appetite moderate.—2/9 Still somewhat lethargic but appetite fairly good. BW 2.35 kg. Killed. ***Autopsy:*** generalized jaundice. Total lack of adipose tissue. Small quantity of clear yellow fluid in abdominal cavity. Bile ducts considerably dilated. Liver enlarged, firm and tough. Otherwise N.A.D. PW 5.65 g (D 3.85 g, L 1.80 g).—*Micr. exam.:* liver, marked biliary cirrhosis; small quantity of fat in cirrhotic connective tissue. No fat in liver cells.

R. 214. Male. 1st obs. day 10/8 1954. BW 2.42 kg.—12/8 Operation: *Ligation of common bile duct.* BW 2.33 kg. Anaesthetic: ether.—14/8 Condition and appetite good.—27/8 Distinct jaundice; condition good.—6/9 Condition still good and appetite fairly good. BW 2.49 kg. Killed. *Autopsy:* generalized jaundice. Total lack of adipose tissue. Moderate quantity of clear yellow fluid in abdominal cavity. Liver yellowish-brown, firm and tough. Bile ducts greatly dilated. Otherwise N.A.D. PW 6.07 g (D 4.17 g, L 1.90 g).—*Micr. exam.:* liver, marked biliary cirrhosis; no fat.

R. 219. Male. 1st obs. day 1/10 1954. BW 2.11 kg.—9/10 Operation: *Ligation of common bile duct.* BW 1.98 kg. Anaesthetic: ether.—11/10 Condition fairly good. Appetite somewhat impaired. —21/10 Fairly acute exacerbation; lethargic, no appetite. In p.m. moribund; killed. BW 1.82 kg. *Autopsy:* generalized jaundice. Total lack of adipose tissue. Moderate quantity of green turbid fluid in abdominal cavity. Liver enlarged, firm and tough. Bile ducts dilated. Otherwise N.A.D. PW 5.19 g (D 2.99 g, L 2.20 g).—*Micr. exam.:* liver, marked biliary cirrhosis; no fat.

GROUP II A: CATEGORY 2

R. 158. Female. 1st obs. day 13/1 1954. BW 2.29 kg.—22/1 Operation: *Ligation of common bile duct.* BW 2.40 kg. Anaesthetic: ether.—In afternoon, increasing lethargy; died in evening. *Autopsy:* small quantity of turbid fluid in abdominal cavity. Operative area: N.A.D. Cause of death could not be definitely established.—No *micr. exam.* made.

R. 159. Female. 1st obs. day 13/1 1954. BW 2.26 kg.—26/1 Operation: *Ligation of common bile duct.* BW 2.39 kg. Anaesthetic: ether.—28/1 Condition affected; lethargic, eats little.—30/1 Successive exacerbation; died in convulsions in evening. *Autopsy:* BW 2.19 kg. Yellow turbid fluid in abdominal cavity. Haemorrhages in omentum. Liver: large necrotic areas with haemorrhages. Bile ducts moderately dilated.—*Micr. exam.:* perilobular increase in connective tissue of liver and numerous necrotic foci. No microscopic examination of other organs.

R. 162. Male. 1st obs. day 20/1 1954. BW 2.11 kg.—5/2 Operation: *Ligation of common bile duct.* BW 2.35 kg. Anaesthetic: ether.—7/2 Condition affected; does not eat.—8/2 Found dead in morning. *Autopsy:* BW 2.44 kg. Operative area: N.A.D. Bile ducts moderately dilated. On surface of liver capsule, a few small yellow spots; cut surface N.A.D. Other organs: N.A.D.—*Micr. exam.:* a few well defined necrotic areas in liver parenchyma. Kidneys: tubular necrosis.

R. 220. Male. 1st obs. day 1/10 1954. BW 2.22 kg.—9/10 Operation: *Ligation of common bile duct.* BW 2.14 kg. Anaesthetic: ether.—11/10 Condition and appetite fairly good.—12/10 Found dead in morning. *Autopsy:* BW 1.98 kg. Incipient putrefaction. Beside the ligated common duct, an overlooked swab. Otherwise N.A.D. Cause of death could not be definitely established.

R. 221. Male. 1st obs. day 16/10 1954. BW 2.32 kg.—20/10 Operation: *Ligation of common bile duct.* BW 2.46 kg. Anaesthetic: ether.—22/10 Condition affected, no appetite.—23/10 In afternoon, moribund; killed. BW 2.28 kg. *Autopsy:* Operative area N.A.D. Bile ducts dilated. Liver: N.A.D. On cut surfaces of kidneys, considerable decrease in cortical zone, reduced to capsule about 1 mm wide around medulla.—*Micr. exam.:* widespread, bilateral necrosis of renal tubules.

R. 222. Male. 1st obs. day 16/10 1954. BW 2.26 kg.—20/10 Operation: *Ligation of common bile duct.* BW 2.40 kg. Anaesthetic: ether.—22/10 Condition fairly good. Eats.—31/10 Condition poorer; lethargic, no appetite.—1/11 Found dead in morning. *Autopsy:* BW 1.85 kg. Total

lack of adipose tissue. Plentiful greenish-brown fluid in abdominal cavity. Extensive adhesions between liver, gallbladder, common duct, stomach and duodenum. Liver somewhat firmer than normally, otherwise N.A.D.—In view of incipient putrefaction, no *micr. exam.* made.

R. 223. Female. 1st obs. day 16/10 1954. BW 2.56 kg.—22/10 Operation: *Ligation of common bile duct.* BW 2.49 kg. Anaesthetic: ether.—24/10 Condition somewhat affected; no appetite.—28/10 Somewhat livelier.—5/11 Moribund; died in afternoon. *Autopsy:* Plentiful green turbid fluid in abdominal cavity; fibrinous deposits on intestines. Greenish discoloration of liver; cut surface necrotic.—No *micr. exam.* made.

R. 224. Female. 1st obs. day 16/10 1954. BW 2.40 kg.—22/10 Operation: *Ligation of common bile duct.* BW 2.24 kg. Anaesthetic: ether.—24/10 Condition affected; no appetite.—26/10 Somewhat livelier.—8/11 Found dead in morning. *Autopsy:* BW 1.83 kg. Incipient autolysis. Plentiful greenish-brown fluid in abdominal cavity. Rupture of liver parenchyma between two lobes.—*Micr. exam.:* slight perilobular increase in connective tissue of liver, with fatty degeneration. No fat in liver cells.

R. 233. Female. 1st obs. day 9/11 1954. BW 3.21 kg.—15/11 Operation: *Ligation of common bile duct.* BW 2.93 kg. Anaesthetic: ether + 100 mg kemithal.—17/11 Condition good. Eats.—30/11 Distinct jaundice, otherwise N.A.D.—1/12 Glucose tolerance test. BW 2.58 kg. Glucose: 4.1 ml.—4/12 More lethargic; appetite poorer. *Alloxan:* 100 mg/kg = 240 mg.—5/12 Found dead in morning. *Autopsy:* BW 2.42 kg. Incipient putrefaction. Rupture of gallbladder into omentum, in which bile was collected in a saccular dilatation, about 6 cm in diameter.—Alloxan effect could not be evaluated. No *micr. exam.* made.

R. 234. Female. 1st obs. day 9/11 1954. BW 3.25 kg.—15/11 Operation: *Ligation of common bile duct.* BW 3.11 kg. Anaesthetic: ether + 150 mg kemithal.—17/11 Condition and appetite good.—18/11 Acute exacerbation in p.m.; lethargic, no appetite.—19/11 Found dead in morning. *Autopsy:* BW 2.82 kg. Incipient putrefaction. Fairly plentiful greenish-brown, turbid fluid in abdominal cavity. Bile ducts dilated. Liver firm and tough; on cut surface, occasional small white spots, presumably necroses.—No *micr. exam.* made.

R. 163. Male. 1st obs. day 20/1 1954. BW 2.04 kg.—11/2 Operation: *Ligation of common bile duct.* BW 2.33 kg. Anaesthetic: ether.—13/2 Condition and appetite fairly good.—2/3 Glucose tolerance test. BW 2.15 kg. Glucose: 3.4 ml.—15/3 Condition consistently good, appetite normal, no jaundice. BW 2.48 kg. Killed. *Autopsy:* no jaundice; adipose tissue normal. Internal organs: N.A.D. Recanalization was found to have taken place between ligated common duct and duodenum; experiment therefore unsuccessful. PW 4.56 g (D 2.98 g, L 1.58 g).—*Micr. exam.:* liver, N.A.D.

GROUP IV C: CATEGORY 1

R. 115. Female. Pair-fed control to R. 114. 1st obs. day 5/5 1953. BW 1.70 kg.—13/5 Glucose tolerance test. BW 1.75 kg. Glucose: 2.8 ml.—15/5 (op. day: R. 114) BW 1.79 kg.—11/6 Glucose tolerance test. BW 2.28 kg. Glucose: 3.7 ml.—14/6 BW 2.24 kg. Killed. *Autopsy:* N.A.D. PW 4.02 g (D 2.55 g, L 1.47 g).

R. 127. Female. Pair-fed control to R. 126. 1st obs. day 29/5 1953. BW 1.55 kg.—5/6 Glucose tolerance test. BW 1.69 kg. Glucose: 2.7 ml.—30/6 BW 2.36 kg.—26/8 BW 3.05 kg.—2/9 (op. day: R. 126) BW 3.14 kg.—25/9 Glucose tolerance test. BW 3.50 kg. Glucose: 5.6 ml.—28/9 BW 3.36 kg. Killed. *Autopsy:* N.A.D. PW 5.60 g (D 4.27 g, L 1.33 g).

R. 157. Male. Pair-fed control to R. 156. 1st obs. day 13/1 1954. BW 2.33 kg.—20/1 (op. day: R. 156) BW 2.45 kg.—4/2 Glucose tolerance test. BW 2.71 kg. Glucose: 4.3 ml.—11/2 BW 2.91 kg. Killed. *Autopsy:* N.A.D. PW 4.67 g (D 3.85 g, L 0.82 g).

R. 161. Male. Pair-fed control to R. 160. 1st obs. day 20/1 1954. BW 2.27 kg.—3/2 (op. day:

R. 160) BW 2.46 kg.—23/2 Glucose tolerance test. BW 2.73 kg. Glucose: 4.4 ml.—28/2 BW 2.85 kg. Killed. ***Autopsy:*** N.A.D. PW 3.58 g (D 2.08 g, L 1.50 g).

GROUP IV E: CATEGORY 1

R. 169. Female. 1st obs. day 23/2 1954. BW 2.09 kg.—4/3 BW 2.45 kg. ***Period of undernutrition*** started: no food every other day; half normal ration other days (water ad libitum throughout).—9/4 Glucose tolerance test. BW 2.28 kg. Glucose: 3.7 ml.—22/4 Glucose tolerance test. BW 2.24 kg. Glucose: 3.6 ml.—6/5 Condition unaffected despite lengthy undernutrition. No food during past 3 days (water ad lib.). BW 2.20 kg. Killed. ***Autopsy:*** internal organs fairly small; no other specific changes. Normal appearance of adipose tissue subcutaneously, in omentum and posterior abdominal wall. PW 3.04 g (D 1.89 g, L 1.15 g).

R. 170. Female. 1st obs. day 23/2 1954. BW 2.16 kg.—4/3 BW 2.43 kg. ***Period of undernutrition*** started (same as in R. 169).—9/4 Glucose tolerance test. BW 2.33 kg. Glucose: 3.7 ml.—6/5 Condition unaffected despite lengthy undernutrition. No food past 3 days. BW 2.36 kg. Killed. ***Autopsy:*** internal organs fairly small; no other specific changes. Appearance of adipose tissue normal throughout. PW 3.68 g (D 2.44 g, L 1.24 g).

R. 171. Male. 1st obs. day 23/2 1954. BW 2.63 kg.—4/3 BW 2.57 kg. ***Period of undernutrition*** started (same as in R. 169).—13/4 Glucose tolerance test. BW 2.35 kg. Glucose: 3.8 ml.—22/4 Glucose tolerance test. BW 2.33 kg. Glucose: 3.7 ml.—10/5 Condition unaffected. No food for past 3 days. BW 2.34 kg. Killed. ***Autopsy:*** internal organs fairly small; no other specific changes. Appearance of adipose tissue normal throughout. PW 2.90 g (D 2.17 g, L 0.73 g).

R. 172. Male. 1st obs. day 23/2 1954. BW 2.61 kg.—4/3 BW 2.65 kg. ***Period of undernutrition*** started (same as in R. 169).—13/4 Glucose tolerance test. BW 2.45 kg. Glucose: 3.9 ml.—6/5 Glucose tolerance test. BW 2.33 kg. Glucose: 3.7 ml.—10/5 Condition unaffected. No food for past 3 days. BW 2.28 kg. Killed. ***Autopsy:*** internal organs fairly small; no other specific changes. Appearance of adipose tissue normal throughout. PW 2.58 g (D 1.61 g, L 0.97 g).

POSTOPERATIVE COURSE

Observation Period

In the operated animals in category 1, the mean duration of the postoperative period was 23.5 days, the figures for the individual animals being 27, 26, 27, 24, 20, 20, 23, 34, 21, 25 and 12 days. In group IV C, the corresponding mean was 26.0 days and the individual figures 30, 27, 22 and 25 days. The observation period in the operated animals in category 2 amounted to a mean 10.2 days after operation, the figures for the individual animals being 0, 4, 3, 3, 3, 12, 14, 17, 20, 4 and 32 days.

As seen in the animal records, the animals in group IV E were underfed; every other day they were given water only, and every other day they received half the standard laboratory diet (*cf.* Chapter 2, p. 35). The object of this undernutrition was to produce a fall in weight of the same order of magnitude as that in group II A. This nevertheless required a considerably longer time than in the operated group. Thus, the mean length of the undernutrition period in group IV E was 65.0 days, and 63,

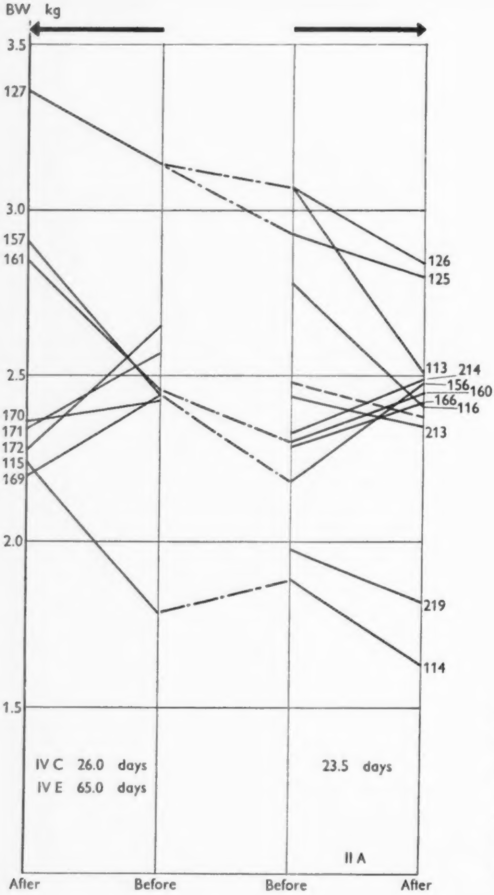

Fig. 25. Change in BW during post-operative observation period: group II A (ligation of common bile duct) and groups IV C and IV E (controls)
— — — — mean value.
—·—·—· paired animals.
Before = before operation.
After = after operation.

63, 67 and 67 days, respectively, in the individual animals. Since the appetite of the operated animals was generally poorest during the last few days of the observation period, the animals in group IV E were given no food at all on the last three days before they were killed.

General Condition

The condition of the operated animals varied appreciably. It was better in category 1 than in category 2. Thus, some animals in category 1 (*e. g.* R. 113, 116 and 166) were completely unaffected during the greater part of

the experimental period. Others (*e. g.* R. 213, 214 and 219) were, it is true, relatively lively but their appetite was distinctly impaired all the time.

All the operated animals naturally exhibited progressive jaundice, and the bilirubin reaction in the urine was strongly positive.

Three animals in category 1 (R. 114, 125 and 126) died spontaneously, but autopsy could be performed soon after death and no post mortem changes were present. All the other animals in category 1 were killed. In category 2, all the animals died spontaneously, except R. 163, the animal in which recanalization had taken place. In 5 cases (R. 222, 223, 224, 233 and 234) the cause of death was cholascos (effusion of bile into the peritoneal cavity) owing to rupture of the gallbladder or bile ducts. In 2 cases (R. 159 and 162) death was presumed to be caused by degenerative, necrotic changes in the liver. In one of these animals, circumscribed necrotic areas were also present in the renal tubules. In an additional case (R. 221) there was extensive necrosis of the renal cortex. No definite cause of death could be established in 2 cases (R. 158 and 220).

Body Weight

The postoperative changes in BW in category 1 are recorded in Fig. 25. In 7 animals there was a loss of weight and in 4 a gain, implying a mean loss for the whole group of 4.9 g/24 hrs. A gain in weight was recorded in all the animals in group IV C, the mean being 14.9 g/24 hrs, whereas all those in group IV E lost weight (mean: 3.5 g/24 hrs).

GLUCOSE TOLERANCE TESTS

The results of the glucose tolerance tests are given in Tables I: 5—I: 7 and Figs. 26 and 27. In group II A, the test was made in 7 animals (6 in category 1 and 1 in category 2) a mean 18.1 days after operation. The mean value of the tolerance area was 6,344 $\pm$1,049 mg-min, and when this value was tested against the mean value in the "normal group", a probably significant difference was obtained (P $\sim$ 0.02): Table 26. However, as may be inferred from Fig. 26, a distinctly increased tolerance area was present in only 3 out of the 7 tests (R. 116, 233 and 166), so that there was considerable scattering in the group. Since, for this reason, the *t* test could not be considered to reflect the conditions accurately, the ranking method of WILCOXON was used for a comparison between the groups. No significant difference between them was found by this means.

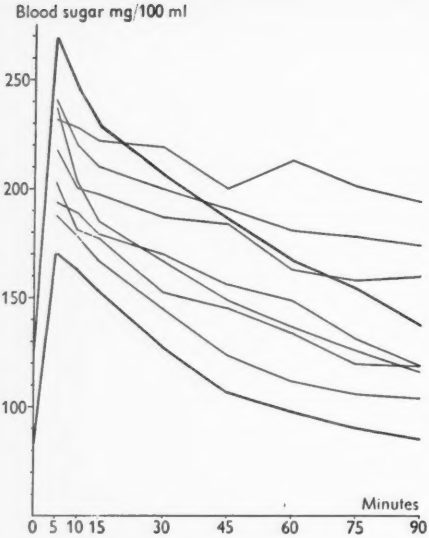

Fig. 26. Glucose tolerance curves: group II A after operation.

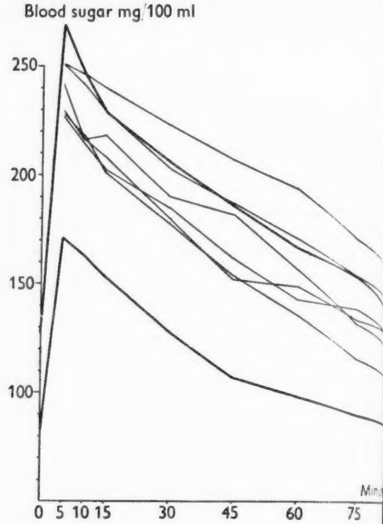

Fig. 27. Glucose tolerance curves: group IV after undernutrition period.

In group IV C, glucose tolerance tests were made a mean 21.5 days after operation of the respective paired animals. The results were entirely within the normal range of variation, the mean value for the tolerance area being 3,853 ±1,125 mg-min. As regards group IV E, the effect of undernutrition on the intravenous glucose tolerance curve was tested not only in the four animals comprising this group, but in an additional two: R. 113 and R. 116. These animals were underfed for 29 days in the same way as those in group IV E proper. As seen from the animal records, they were subsequently transferred to group II A. They were not operated on until more than two months after the end of the period of undernutrition. In the interim, they were given the standard diet. It therefore seems probable that any effects of undernutrition would have disappeared during this time.

A glucose tolerance test was made in these total 6 underfed animals after a mean 38.4 days of undernutrition. As may be inferred from Table I: 7 and Fig. 27, a tendency to decreased glucose tolerance was present, the mean tolerance area being 7,015 ±692 mg-min. Comparison of this mean value with that of the "normal group" showed a highly significant difference ($P \sim 0.001$), (Table 26). Using the ranking method of WILCOXON, the difference was significant ($P \sim 0.01$).

AUTOPSY

General Observations

In all the animals in category 1, generalized, intense icteric coloration of the tissues was observed. The bile ducts and gallbladder were greatly dilated, with thickened walls, and in most cases contained dark, viscous bile under strong pressure. Ascites was a common finding. In 5 animals (R. 113, 114, 116, 213 and 214) the fluid was clear and yellow and the quantity was moderate, but in 4 cases (R. 125, 126, 160 and 219) there was a large quantity of green or yellowish-brown, turbid fluid in the peritoneal cavity, *i. e.*, cholascos. Two animals (R. 156 and 166) had no fluid in the peritoneal cavity. In every case the liver was enlarged, firm, tough and cirrhotic.

As in group I A, there was a considerable reduction of the *adipose tissue* in most cases. This feature was even more marked than in the former group. Thus, in 7 cases no fat whatsoever could be seen macroscopically. In 2 other cases the fat reduction was almost complete and in 1 it was considerable, although not complete. Only in one animal (R. 156) was the adipose tissue on the whole of normal appearance. Microscopic examination of the mesenchymal tissue at the former site of the adipose tissue showed the same features as in group I A (Fig. 23).

In the control groups, the adipose tissue had a normal appearance. Although in the underfed group IV E the internal organs were small and atrophic, fairly plentiful adipose tissue was present in the posterior abdominal wall, perirenally and in the omentum. Microscopic examination of such tissue showed a completely normal picture, with no reduction in the intracellular fat content.

Pancreas Weight

The pancreas weight was greater in group II A (category 1) than in the control groups, despite the fact that the amount of interstitial fat was less in the former group (Table 10). Thus, the mean value of PW was 6.89 g in group II A, 4.47 g in group IV C and 3.05 g in group IV E. Statistical comparisons between these means showed the difference between groups II A and IV C to be probably significant ($P \sim 0.02$) and that between groups II A and IV E to be highly significant ($P \sim 0.001$), but also a probably significant difference between the control groups ($P \sim 0.05$). If, however, a comparison is made between the means of PW/BW (II A: 2.93 g, IV C: 1.58 g, IV E: 1.33 g), the degree of significance between groups II A and

8

IV C increases (P $\sim$ 0.01), that between II A and IV E remains unchanged, but no significant difference is any longer present between groups IV C and IV E (P $\sim$ 0.1). The reason for the last-mentioned condition is the difference between the body weight in groups IV C and IV E. As stated earlier, the animals in the latter group lost weight during the experimental period, whereas those in the former gained weight. The total reduction in BW had thus influenced the PW as well.

QUANTITATIVE MICROMORPHOLOGIC ANALYSES

Size of Total Pancreas Parenchyma

The size of the total pancreas parenchyma (V_p) was also greater in group II A than in the control groups (Table 10). The mean value was 1,600.2 mm³ in group II A, 1,105.4 mm³ in group IV C, and 1,123.2 mm³ in group IV E, *i. e.*, no appreciable difference between the last two groups. Statistical analyses of the differences between groups II A and IV C and between groups II A and IV E showed a probably significant difference in both cases (P $\sim$ 0.02). When IV C and IV E were assembled into one group, the mean was 1,114.3 mm³; comparison between this value and the mean for group II A showed a highly significant difference (P $\sim$ 0.001).

Calculation of V_p/BW gave the following means: 685.9 mm³ in group II A, 399.0 mm³ in group IV C and 489.7 mm³ in group IV E. There is no significant difference between groups IV C and IV E (P $\sim$ 0.1), that between groups II A and IV C is significant (P $\sim$ 0.01) and that between groups II A and IV E is probably significant (P $\sim$ 0.05).

The common mean of V_p/BW for groups IV C and IV E was 444.3 mm³; the difference between it and the mean for group II A is highly significant (P $\sim$ 0.001).—See Table 9.

Size of Islet Tissue

The results of the quantitative analyses of the islet tissue are recorded in Table 11. In the operated group, the size of the islet tissue was greater than in the control groups, although the differences were of varying magnitude for n_i and m_i, respectively. Thus, the mean value of n_i was 2,468.9 in group II A, 1,770.5 in group IV C and 1,404.5 in group IV E. The difference between groups II A and IV C is not significant (P $\sim$ 0.1), whereas that between groups II A and IV E is significant (P $\sim$ 0.01). As in the PW

the difference between groups IV C and IV E is probably significant (P $\sim$ 0.05). When the number of islets is given per kg of body weight, n_i/BW, the following means are obtained: 1,046.2 in group II A, 627.0 in group IV C and 610.8 in group IV E. A comparison between these values gives a significant difference between groups II A and IV C (P $\sim$ 0.01) and between groups II A and IV E (P $\sim$ 0.01) but, similarly to the conditions in analysis of PW/BW, no difference between groups IV C and IV E (P $\sim$ 0.7).

The mean value of m_i was 3,709.8 μ^2 in group II A, 2,950.8 μ^2 in group IV C and 3,214.8 μ^2 in group IV E. This implies a probably significant difference between groups II A and IV C (P $\sim$ 0.05), but no difference between groups II A and IV E, nor between groups IV C and IV E.

The mean value of the total islet volume, V_i, was 11.04 mm³ in group II A, 6.34 mm³ in group IV C and 5.39 mm³ in group IV E. Here, the difference between groups II A and IV C is probably significant (P $\sim$ 0.05), that between groups II A and IV E significant (P $\sim$ 0.01), but that between groups IV C and IV E is not significant (P $\sim$ 0.4). Finally, the following means were obtained for the islet volume per kg of body weight, V_i/BW: 4.69 mm³ in group II A, 2.21 mm³ in group IV C and 2.35 mm³ in group IV E. This implies a significant difference between groups II A and IV C (P $\sim$ 0.01) and between groups II A and IV E (P $\sim$ 0.01) but, obviously, no significant difference between groups IV C and IV E.

If the control groups are assembled in one group, the common mean of V_i is 5.86 mm³, and the common mean of V_i/BW is 2.28 mm³. The differences between these means and the corresponding means in group II A are highly significant in both cases (P $\sim$ 0.01). — See Table 9.

Alpha and Beta Cell Count

The results of the alpha and beta cell count (Tables IV: 3—IV: 5) showed no significant difference between the three groups. The mean alpha cell incidence was 16.76 per cent in group II A, 15.66 per cent in group IV C and 19.54 per cent in group IV E. In two cases in group II A, the figures differed appreciably from those for the rest of the group. Thus, the alpha cell incidence was only 4.63 per cent in R. 156, and 28.88 per cent in R. 126. No difficulties were encountered in distinguishing between the alpha and beta cells in these extreme cases. It is therefore improbable that the discrepancies are to be ascribed to the technique.

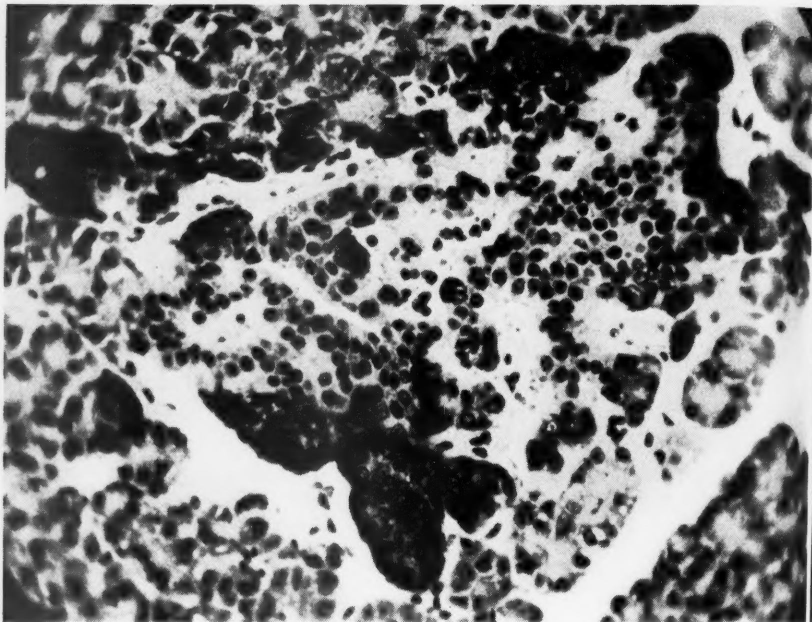

Fig. 28. Pancreas: biliary stasis animal (R. 214, group II A). Typical large islet with high vascularity and nuclear polymorphism. Gomori stain. Magnification 500 X.

HISTOLOGIC AND HISTOCHEMICAL EXAMINATION

Pancreas

The *general appearance* of the exocrine pancreatic tissue was the same in the operated animals as in the controls, except that the parenchyma looked more compact in the former, owing to the often considerable reduction in adipose tissue. Otherwise, the acini, vessels and ducts had a normal appearance. In addition to the quantitative changes described, the same high vascularity and nuclear polymorphism were seen as in group I A. Fig. 28 shows an islet from an animal in group II A, killed 25 days after operation.

Islet haemorrhage was observed in two animals (R. 213 and 214). In some islets the haemorrhage was considerable. The greater part of the islet tissue was then occupied by blood corpuscles, so that the islet cells were displaced peripherally, where they were found as a thin rind of epi-

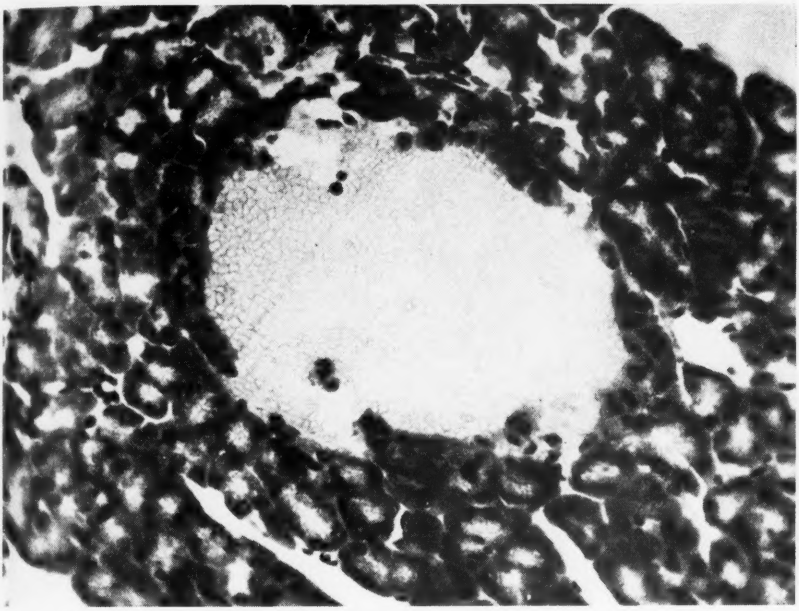

Fig. 29. Pancreas: biliary stasis animal (R. 214, group II A). Islet haemorrhage. Gomori stain. Magnification 300 X.

thelial cells. In other islets, haemorrhage was less severe and had then caused only slight separation of the islet cell cords. No haemorrhages in the acinar tissue were visible in these cases. Nor did these animals exhibit any signs of a generalized haemorrhagic diathesis. Figs 29, 30 and 31 show examples of such intra-islet haemorrhage.

Other Organs

Microscopic examination of the *liver* showed, as could be expected, cirrhotic changes of the biliary type. In most cases they were severe, with considerable increase in the perilobular fibrous tissue, separation of the lobules and derangement of the normal hepatic structure. Only in one case (R. 160) was the increase in connective tissue less marked, and only inappreciably encroached on the normal parenchyma.

The *lipid content* of the *liver cells* was low in all animals in group II A (Table 12). The semi-quantitative grading was as follows: 0, 0, 0, 1, 0, 2,

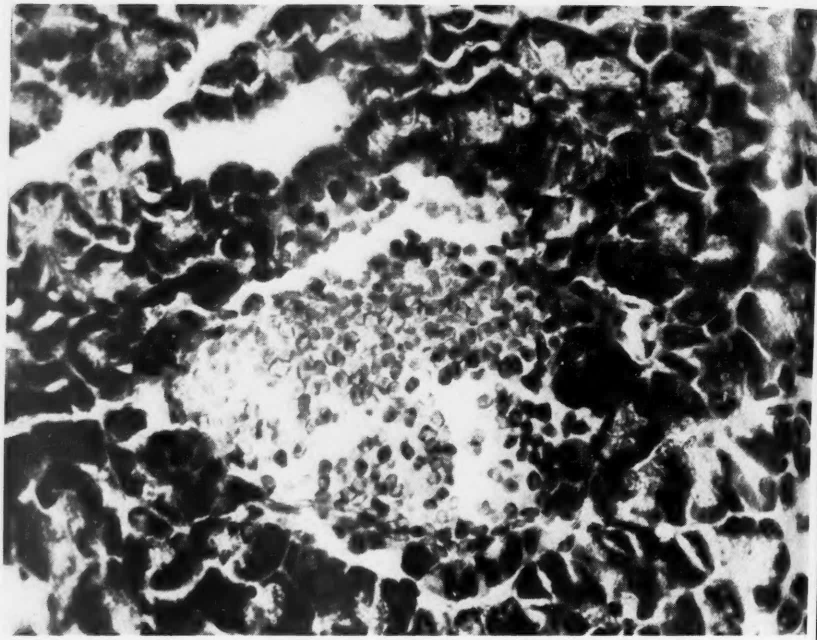

Fig. 30. Pancreas: biliary stasis animal (R. 213, group II A). Islet haemorrhage. Gomori stain. Magnification 400 X.

0, 0, 0, 0, 0. The corresponding figures in group IV C were 6, 6, 1, 5, and in group IV E, 4, 3, 2, 3. The average was thus 0—1 in group II A, 4—5 in group IV C, and about 3 in group IV E. Moderate quantities of large intracellular fat droplets were found in the newly-formed cirrhotic connective tissue in some cases in group II A.

The *glycogen content* of the *liver cells* was also low in the operated group, *i. e.*, 0, 5, 0, 3, 0, 2, 4, 0 (the animals that died spontaneously having been excluded). The corresponding figures in group IV C were 5, 6, 7, 7, and in group IV E, 3, 4, 2, 1. This corresponds to an average of 1—2 in group II A, 6—7 in group IV C and 2—3 in group IV E.

The *alkaline phosphatase activity* in the *liver cells* was graded as follows. Group IV A: 4, 2, 4, 2, 3, 0, 6, 4, 5, 6, 7, group IV C: 1, 5, 5, 6, and group IV E: 6, 6, 6, 6. The average was thus 3—4 in group IV A, 4—5 in group IV C and about 6 in group IV E. These results have not been interpreted as constituting any definite difference between the groups. In group II A,

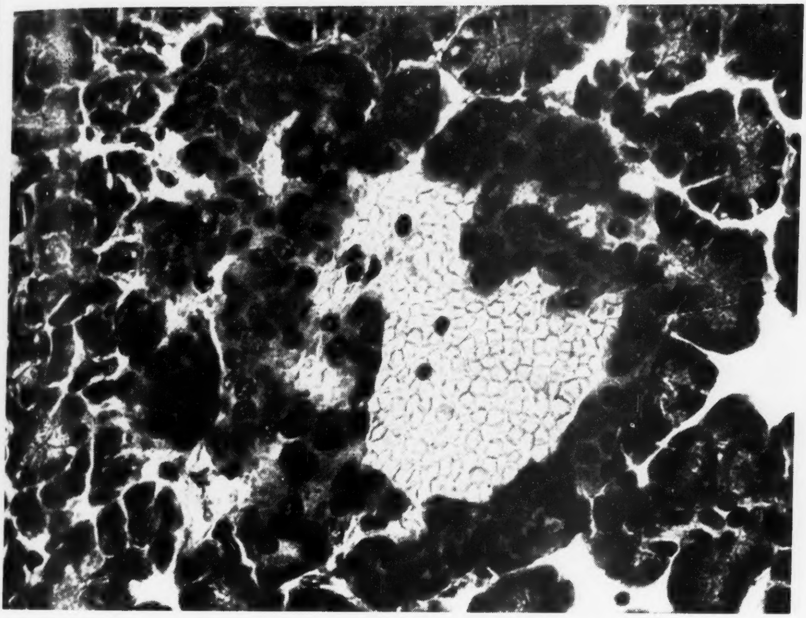

Fig. 31. Pancreas: biliary stasis animal (R. 213, group II A). Islet haemorrhage. Gomori stain. Magnification 490 X.

phosphatase activity was present in the undamaged parenchyma only, and thus not in the perilobular areas with cirrhotic changes.

The *phosphatase activity* of the *duodenal mucosa* was high in all the groups. The *lipid content* of the *adrenocortical cells* was also normal in each of the groups.

C. DISCUSSION

POSTOPERATIVE COURSE

The following inferences could be drawn in a comparison between the results of the two interventions described in this chapter. In both types of interference with the bile flow, it is possible to obtain for microscopic analyses animals that have survived for three to four weeks. As far as the biliary fistula experiments are concerned, it has earlier been believed—as

pointed out by BISSELL & ANDREWS, among others—that complete external biliary drainage was invariably and rapidly fatal, and that this effect was directly associated with the loss of bile. The same authors were able to demonstrate that this view is incorrect, and that the high mortality is rather to be ascribed to mechanical or infectious complications of the operation than to the loss of bile. COLWELL has also stressed, as his experience in rats, that death of biliary fistula animals is more often due to complications of a technical nature than to metabolic disturbances.

The same experience was made in the present investigation. Provided that loss of fluid and electrolytes was compensated, I was able to keep rabbits with a biliary fistula alive and in relatively good condition for several weeks. Most of the animals that died at an early stage exhibited signs of infection (cholangitis) or complications of a technical or mechanical nature (leakage of bile or strangulation ileus).

The conditions naturally differed in ligation of the common bile duct without drainage of the bile, as in group II A. Of the 21 animals operated on (the animal in which recanalization of the common duct took place being excluded), 13 died spontaneously. Six of them died during the first 10 days after operation, 5 during the subsequent 10 days, and 2 on the 24th and 26th day, respectively. Of the remaining animals, three were moribund when they were killed on the 12th, 23rd and 27th postoperative day, respectively. Thus, only five animals were in relatively good condition at the end of the experimental period, 20 to 30 days after operation. In most cases, the cause of death was either cholascos or degenerative, necrotic lesions of the liver or kidneys.

EDLUND had about the same mortality rate in a series of rats in which the common duct had been ligated. In dogs, the survival time seems to be longer and also to be dependent on the nature of their diet. Thus, BOLLMAN has stated that dogs with total biliary obstruction can survive for about a year if they are given a high-carbohydrate diet, but that they die within a week if a meat diet is given. Similar experience in biliary fistula dogs has been reported by WHIPPLE. The survival time also seems to be relatively long in human subjects with biliary obstruction. Children with congenital atresia of the bile ducts have been reported to live for several years (LICHTMAN), and RAPOPORT mentioned a patient who survived for 9½ years with the help of antibiotics and vitamin K therapy. In such cases it nevertheless seems justified to question whether the obstruction was really complete.

In my material, both types of operation were associated with a loss of weight. This was, on the average, more conspicuous in the biliary fistula

group, owing to the presence of several animals with infectious hepatic complications, in which the weight loss was considerably greater than in the uncomplicated cases (Figs. 18 and 25).

GLUCOSE TOLERANCE

The results of the glucose tolerance tests in the two groups of operated animals can be summarized as follows. If any deviation from the normal tolerance did, in fact, take place, the tendency in group I A was to an increased tolerance, and that in group II A to a decreased tolerance. Decreased tolerance was found in the controls in groups IV B and IV E, *i. e.*, a reflection of the established fact that starvation—in similarity to a high-fat diet—decreases the glucose tolerance, and gives the tolerance curves a diabetic stamp: "hunger diabetes" (SWEENEY; HIMSWORTH; BEST & TAYLOR). In group IV C, the glucose tolerance curves exhibited no noteworthy features. As pointed out earlier, the degree of undernutrition was not marked in this group.

Thus, the expected effect of undernutrition on the glucose tolerance failed to appear in 6 out of the 7 biliary fistula animals. Moreover, these six animals showed a tendency to the opposite effect. The effect of undernutrition was lacking in 4 out of the 7 animals with biliary stasis, but no shift in the opposite direction occurred in these four cases. Decreased glucose tolerance was thus present in altogether four animals in the two groups (R. 129, 116, 233 and 166). It was even more conspicuous than in either of the underfed control groups. The mean value of the tolerance area in the four animals in question was 9,341 mg-min, whereas that in group IV E was 7,015 and that in group IV B was 5,408 mg-min. In all four cases there were definite indications of liver damage.

It is obviously impossible to draw any definite conclusions from these results, particularly in view of the relatively small number of tests. The complex nature of the blood-sugar regulating mechanisms (*cf.* MCQUARRIE) makes it difficult to determine in the individual case which factor or factors are responsible for a certain type of tolerance curve. Against the background of the present material there are, however, two factors that warrant a comparative analysis, *i. e.*, starvation, and liver damage.

Both these conditions have a common factor, namely, a decrease in the liver glycogen content. As SWENSSON has pointed out, "it is a generally recognized and accepted fact that starvation tends to lower the liver glycogen content", a fact that he was able to confirm in experiments on

mice. As far as liver damage is concerned, there is plentiful evidence to show that a damaged liver contains little glycogen (*cf.* LICHTMAN; SOSKIN & LEVINE). EDLUND found both low liver glycogen and decreased blood sugar in rats with total biliary stasis. In my material as well, a low grade of liver glycogen was found in the animals with biliary stasis (group II A), in addition to the underfed animals (group IV E): see Table 12.

SOSKIN has emphasized that decreased glucose tolerance is often found in diseases of the liver, and that it is possible with the intravenous glucose tolerance test to differentiate between the "true" diabetic type of curve and that occurring in liver damage. EDLUND, using this test, also found a diabetic type of glucose tolerance in a series of patients with biliary stasis. CONN & SELTZER stressed, in a discussion of hepatogenic hypoglycaemia, that such patients with hepatic lesions often exhibit a diabetic type of glucose tolerance curve. The same view was expressed by COLLER & JACKSON. CONN & SELTZER suggested impairment of hepatic glycogenesis as well as of hepatic glycogenolysis as a probable explanation of these findings. A similar interpretation was given by SOSKIN & LEVINE. EDLUND, on the contrary, suggested impairment of glycogenesis combined with *increased* glycogenolysis as the cause, although the latter appears less likely, and is also unnecessary to assume. GEILL's experience of a smaller rise in blood sugar after administration of adrenalin in patients with liver disease than in normal subjects also argues against this assumption.

Although the glycogen content of the liver is low both in starvation and in liver damage, it is probable that the mechanism underlying the low glucose tolerance is not the same in both cases. Opinions are, however, at variance with respect to this mechanism. SOSKIN & LEVINE have stated that, in starvation, the lack of preformed carbohydrate forces the liver to "make all the necessary carbohydrate from its own resources", which implies a greater activity than normally in the liver. Consequently, when glucose is administered, the liver requires a longer time for deceleration of its sugar output than normally, which results in the "diabetic" type of curve. BEST & TAYLOR, on the other hand, refer to investigations showing that the insulin content of the pancreas is considerably reduced in starved animals (BEST, HAIST & RIDOUT), and suggest that the cause of decreased glucose tolerance may be depressed insulin liberation. Other causative factors can also be envisaged. For example, HIMSWORTH & SCOTT have shown that the tolerance-reducing effect of a low-carbohydrate diet disappears after hypophysectomy.

Consequently, the low glucose tolerance found in the four animals in the present investigation (R. 129, 116, 233 and 166) may have been a

result either of their liver damage, or of their state of undernutrition, or of both factors in combination. The reason why the other four animals in group II A, who also had liver damage, did not exhibit any decrease in glucose tolerance is uncertain. This also applies to the six animals in group I A in which the glucose tolerance was inclined to be raised, despite undernutrition. A possible explanation is given by the morphologic findings in the pancreas. The increase in islet tissue may have caused an increased insulin output, which could have counteracted the effect of undernutrition on the glucose tolerance. Another, although highly hypothetical, explanation is a steroid imbalance resulting from the biliary fistula operation. For, Bissell & Andrews have stated that they found a "disordered internal metabolism of sterols which inhibits proper utilization of them" in dogs with a biliary fistula.

Evaluation of the phosphatase activity in the liver threw no light on the matters just discussed, since the degree was about the same in all the groups (*cf.* Table 12). The method used is probably not sufficiently sensitive to reflect any variations in the phosphatase content that may be associated with altered glycogenolysis in the liver. Similar results were obtained by Wachstein & Zak with the same method in experimental biliary cirrhosis in rabbits. In any event, the problem of phosphatase activity in obstructive jaundice is beyond the scope of the present investigation.

LOSS OF ADIPOSE TISSUE

A general finding in both groups was a more or less complete reduction in all adipose tissue. Microscopic examination showed marked atrophic changes in this tissue, similar to those seen in serous adipose tissue atrophy in man. The liver cells were also more or less completely devoid of stainable fat, in contrast to the similarly underfed controls. If the reduction in body weight is taken as a gauge of the degree of undernutrition, no constant relation exists between the loss of adipose tissue and the degree of undernutrition. For example, there was a total lack of macroscopically visible fat in R. 166 and R. 214 in group II A, although both animals had gained weight during the postoperative period. In the control groups, no loss of adipose tissue was observed, even in group IV E in which the undernutrition factor was most pronounced. Nor it is probable that the operation itself was responsible for the reduction in adipose tissue. For, no loss of fat was noted in animals that underwent other operations,

i. e., ligation of the pancreatic duct (group III) or ligation of the left ureter (group IV D).

This phenomenon of "fat starvation" has been discussed by BISSELL & ANDREWS, among others. They were, however, unable to confirm certain earlier statements regarding a specific loss of fat in biliary fistula dogs. In some of their animals, even obesity, alimentary lipaemia and fatty infiltration of the liver were observed. It is thus evident from their investigation that although it is unquestionable that the bile plays a role "in every step of emulsification, digestion and absorption of lipids" (LICHTMAN), a certain amount of fat may be absorbed even in the absence of bile from the intestinal tract.

It is therefore possible that the loss of adipose tissue in my animals in groups I A and II A was not directly associated with a decrease in fat absorption owing to cessation of the bile flow to the intestine. Further support is lent to this hypothesis by the fact that the purely vegetarian diet given contained only an inappreciable quantity of fat (p. 59), in contrast to the food given to BISSELL & ANDREWS' dogs.

SCHOENHEIMER has shown by isotope experiments that, in the normal organism, lipogenesis from carbohydrates is a constant, continuous process, and that processes of breaking down and resynthesis take place without interruption in the adipose tissue. Using a similar technique, STETTEN & BOXER were able to show that, in the rat, "at least 10 times as much of the dietary glucose was used to synthesize fatty acids as was used to synthesize glycogen". In view of these investigations, it does not appear likely that maintenance of the fat depots in the intact rabbit depends on the inappreciable fat content of the diet.

On the basis of the aforementioned observations, the following tentative hypothesis can be put forward to explain the absence of adipose tissue in rabbits with suppression of the bile flow to the intestine. The diminution, but probably not complete inhibition, of intestinal absorption of the quantitatively inappreciable dietary fat is not responsible for the loss of adipose tissue. Breaking down of fat in the depots takes place to the normal extent, but resynthesis of fat from carbohydrate sources has been impaired. Since lipogenesis is believed to be most active in the liver (*cf.* PETERSEN; HAAGENSEN), it is conceivable that the disturbance in fat synthesis is a direct result of damage to the liver cells caused by the relevant operations. That such damage exists in total biliary stasis, as in group II A, is unquestionable (EDLUND). In the biliary fistula animals, on the other hand, it has not been possible with the methods of investigation used to demonstrate with certainty any morphologic basis of such supposed liver cell damage.

SIZE OF PANCREAS

The pancreas was larger in the groups of operated animals than in the respective control groups. This difference was apparent both in determinations of the weight of the organ (PW and PW/BW) and of the size of the parenchyma (V_p and V_p/BW). The weight expresses the size both of the actual parenchyma and of the adjacent non-parenchymal structures, mainly the adipose tissue. The last-mentioned had undergone considerable generalized reduction in the operated animals; consequently, the difference between the weight in the respective groups lends additional support to the view that an increase in the size of the pancreas parenchyma had, in fact, taken place.

Fig. 32 illustrates the order of magnitude of V_p/BW in the various groups. They include groups IV A and IV D, *i. e.*, the four entirely untreated animals, and the four in which the left ureter was ligated; all of them were given the full standard diet. (The records of these animals are given in Chapter 7.) There was also a significant difference between these groups and the operated groups I A and II A with respect to V_p/BW, but not with respect to the absolute figures (V_p).

The percentage increase in the parenchymal volume, expressed as V_p/BW, amounted to 69.3 per cent in group I A in relation to IV B, to 54.4 per cent in group II A in relation to groups IV C and IV E collec-

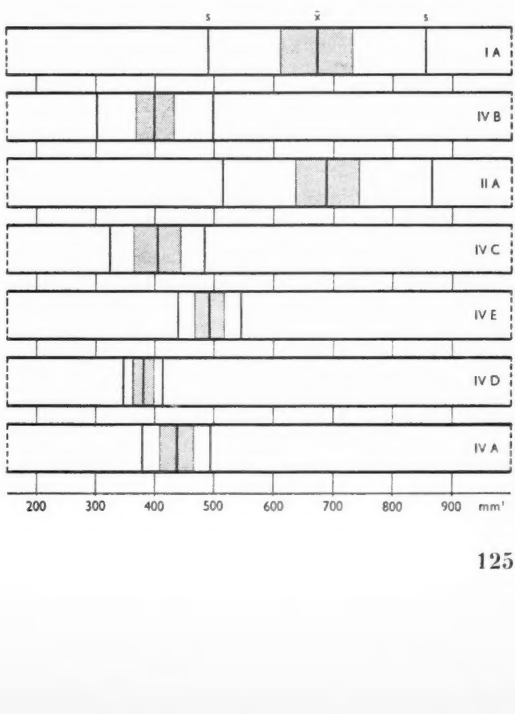

Fig. 32. Pancreas volume (V_p/BW): non-diabetic groups with functioning exocrine parenchyma. Mean values (x) and standard deviation (s). Shaded areas denote $x \pm e(x)$.

tively, and to 62.1 per cent in groups I A and II A combined in relation to groups IV B, IV C and IV E combined.

Table 9 shows a collocation of the statistical comparisons between the different groups. It can be inferred that the differences are probably significant in a comparison between the absolute figures, and that the degree of significance generally increases when a comparison is made between the volume expressed per kg of body weight. In comparison between the combined groups, the differences are highly significant with respect to both the absolute and the relative figures for the volume.

If the change in the V_p had been directly related to the change in BW, the differences between the absolute figures for the volume would naturally have disappeared in comparison between the figures for the volume per kg of body weight. This was the case in a comparison between the pancreas weight in the relatively greatly underfed animals in group IV E with a falling weight curve, and the inappreciably or not at all underfed animals in groups IV C and IV A with a rising weight curve. Here, there was a difference between the absolute figures for the PW but not between those for the PW/BW, this being an expression of the natural fact that a general reduction in body weight is accompanied by a decrease in the pancreas weight. The decrease in weight evidently affects the non-parenchymal parts to the greatest extent since, in a comparison between the figures for the parenchymal volume in these groups, the aforementioned circumstance was less apparent.

It is an accepted fact that inanition is associated with a decrease in the size of the pancreas, even if it is not generally evident from earlier reports whether the parenchymal or the non-parenchymal parts of the organ are affected. According to Jackson, who surveyed the earlier literature on this matter, the pancreas undergoes "marked atrophy" in starvation. In man, the atrophy is stated to be proportional to the total reduction in weight and in adult animals is, as a rule, even greater than the total atrophy. According to Voit (cited by Lovatt Evans), the decrease in pancreas weight amounts to 17 per cent during starvation. Consequently, the lack of a difference between the absolute size of the pancreas in an underfed group of animals and in a normally fed group is also an indication that an increase has taken place in the size of the pancreas parenchyma in the former group.

Fig. 33 shows the relation between the absolute volume of the parenchyma and the postoperative change in weight in the paired animals of groups I A and IV B. The difference between the parenchymal volume in the individual pairs tends to be greater the smaller the difference is

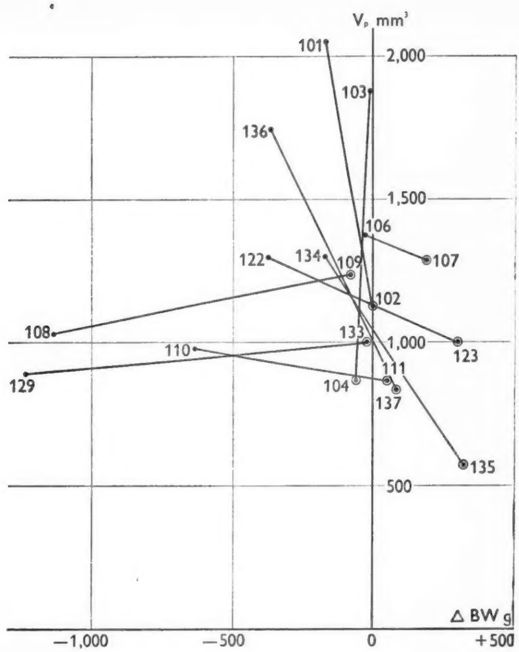

Fig. 33. Pancreas volume (V_p) in comparison to postoperative change in BW: groups I A and IV B.
· = animal in group I A.
⊙ = animal in group IV B.

between the change in weight. In three cases with liver damage in which the postoperative loss of weight was considerable, the inanition effect on the volume of the pancreas parenchyma predominated, and no difference was present between the paired animals with respect to the *absolute* parenchymal volume. A distinct difference between the respective V_p/BW values was, on the contrary, found in these cases.

As far as I have been able to ascertain from the literature, an increase in the total size of the pancreas as a result of interruption of the bile flow to the intestine has not earlier been mentioned. No data regarding the total size of the pancreas are given in the aforementioned works of Bissell & Andrews, of Edlund, of Leriche & Young, or of Verne, among others.

The methods used in the present investigation could obviously not be expected to throw light on the causative mechanism of this enlargement of the pancreas. Nor does earlier knowledge of the influence of bile on the pancreatic juice and on the secretory mechanism of the external pancreatic function provide any explanation, particularly as opinions on these matters are to some extent conflicting. It appears to be generally accepted (Best &

Taylor) that bile or bile salts activate pancreas lipase, "bring out the hidden lipolytic property of the pancreatic juice" (Babkin). Lagerlöf nevertheless found that bile had no effect on the lipase activity *in vitro.* According to Mellanby, the introduction of bile into the duodenum of cats caused a copious secretion of pancreatic juice. In man, Lagerlöf had a similar experience with bile salts which, introduced into the duodenum of a patient with complete obstruction of the bile flow owing to cancer, caused an abundant flow of pancreatic juice. The type of secretion was similar to that obtained after administration of secretin. Mellanby expressed the view that the bile is an essential stimulating agent in external pancreatic secretion, but this view has been rejected by several workers. Thus, according to Dragstedt & Woodbury, the complete removal of bile from the intestine had no effect on the volume of pancreatic juice in dogs. Thomas & Crider (1941) had the same experience and stated that they had been able to show, in both cats and dogs, that bile increases the flow of pancreatic juice only under certain abnormal conditions. In a later investigation, Thomas & Crider (1943) found evidence of an inhibitory effect of bile on the response of pancreatic secretion to various naturally occurring stimuli. According to Ivy, bile is at most an unessential adjuvant to pancreatic secretion. In surveying the literature on the matter, Babkin expressed the view that "bile not only fails to stimulate the pancreatic secretion, but even inhibits it". The fact that the statements are thus somewhat contradictory is presumably to be ascribed partly to the use of essentially different techniques for investigation but partly, as suggested by Lagerlöf, to species differences as well.

If the view that bile has an inhibitory effect on pancreatic secretion should prove correct, it would seem justified to expect increased secretion after interruption of the bile flow to the intestine. Hypertrophy of the pancreas parenchyma as a secondary phenomen would be conceivable. However, such an explanation is naturally completely hypothetical.

SIZE OF ISLET TISSUE

An increase in the size of the islet tissue was found in both the biliary fistula animals and those with biliary stasis. The increase was significant in relation to the values in the control groups in comparison both between the absolute values (V_i) and between the V_i/BW values (Table 9). Expressed in per cent, the increase in V_i/BW amounted to 80.9 per cent in group I A in relation to group IV B, to 105.7 per cent in group II A in

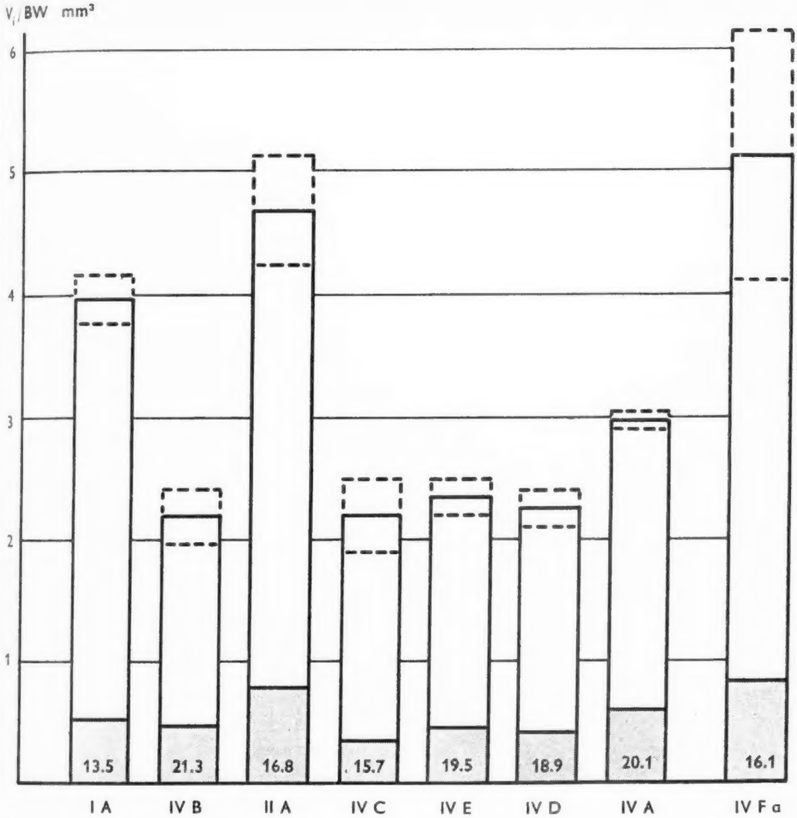

Fig. 34. Islet volume (V_i/BW): non-diabetic groups with functioning exocrine parenchyma. Mean values. – – – – $x \pm e(x)$. Shaded areas and figures in them denote mean alpha cell incidence in respective groups.

relation to groups IV C and IV E combined, and to 96.0 per cent in groups I A and II A collectively in relation to the combined control groups (IV B, IV C and IV E).

The size of the islet volume is shown diagrammatically in Fig. 34. The values for groups IV D, IV A and IV F a are included. It is seen that control group IV D (ligation of left ureter) did not differ in this respect from control groups IV B, IV C and IV E. The islet volume was somewhat larger in the untreated, normally fed control group IV A. In absolute figures (V_i) it does not differ significantly from that in groups I A and II A,

but expressed as V_i/BW there is a significant difference between it and the corresponding value in group I A (P ∾ 0.01), and a probably significant difference between it and the value in group II A (P ∾ 0.05). It can also be inferred from Fig. 34 that the mean islet volume per kg of body weight in group IV F a (animals given glucose) was approximately on the same high level as that in the operated animals (groups I A and II A). There was, however, considerable scattering in group IV F a; consequently, the difference between this group and the normal group IV A is not significant, despite the high mean value in the former group.

Fig. 34 also shows the mean of the alpha cell incidence in the respective groups. In all of them the value is within the normal range (FERNER). Since no separate determination was made of the volume of the individual types of cell in the islets, it is not possible to compare the respective proportions of the alpha and beta cells in the total islet volume. With some approximation, it can nevertheless be stated that the beta cells were mainly responsible for the increase in islet volume in groups I A, II A and IV F a.

The degree of significance was generally higher for the differences be-

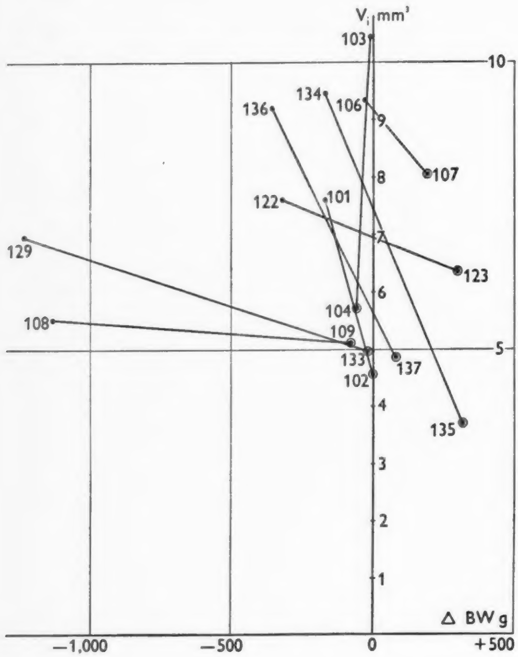

Fig. 35. Islet volume (V_i) in comparison to postoperative change in BW: groups I A and IV B.
• = animal in group I A.
⊙ = animal in group IV B.

tween the values for the number of islets (n_i/BW) than between those for the islet size (m_i). The same tendency was noted by VERNE in his biliary fistula experiments, this "polynésie" being interpreted as a result of new formation of islets.

Fig. 35 shows the relation between islet volume (V_i) and postoperative change in body weight in the paired animals of groups I A and IV B, in the same way as in Fig. 33 with respect to the total volume of the pancreas parenchyma. The tendency is the same, *i. e.*, the smaller the difference between the postoperative weight in the paired animals, the greater is the difference in the total islet volume. Consequently, the present investigation provides no evidence in support of the hypothesis that a state of inanition is associated with an increase in the islet volume (*cf.* p. 30). If inanition has any effect whatsoever on the islet volume, it seems to be in the form of a decrease in size. This is illustrated by a comparison between the well nourished group IV A and the greatly underfed group IV E. In the former, the mean V_i/BW is 2.98 mm³; in the latter it is 2.35 mm³, the difference (0.63 mm³) being significant ($P \sim 0.01$).

The mechanism underlying the change found in the size of the islet tissue, as well as the phenomenon of islet haemorrhage, will be discussed in Chapter 9.

D. SUMMARY

Bile flow experiments were performed on 41 non-diabetic animals. A *biliary fistula* was created in 19 cases, and *ligation of the common bile duct* was performed in 22. In 9 of the former animals, the mean postoperative observation period amounted to 27.6 days; the corresponding mean in 11 animals in the latter group was 23.5 days. Quantitative micromorphologic analyses were performed in these 20 cases, and the results were compared with those in 17 similarly fed controls.

An account is given of the *postoperative course* in these experiments.

Glucose tolerance tests were made before and after the respective interventions. As a rule, the biliary fistula animals exhibited a slightly increased tolerance in comparison to the controls, whereas no change in the tolerance was recorded in the animals with biliary stasis. A distinctly decreased tolerance was, however, recorded in one animal in the former group and in three in the latter, the decrease being greater than in the similarly underfed controls. This decreased tolerance has been interpreted as a result of impaired glycogenesis and glycogenolysis due to liver damage. In

the biliary fistula animals, an increased insulin output from the pancreas *may* have been responsible for the raised glucose tolerance.

At autopsy, a more or less severe degree of reduction in the *adipose tissue* was found in both groups of operated animals. This feature is discussed. It is considered improbable that its cause is decreased absorption of the quantitatively inappreciable dietary fat. It may have been due to a disturbance in lipogenesis, as a result of liver cell damage.

An *enlargement of the pancreas* was observed in both the biliary fistula and the biliary stasis animals. It appeared both in the weight of the organ, and in micromorphologic determination of the parenchyma; significant differences were present in relation to the corresponding values in the control groups. The increase in parenchymal volume, expressed in mm^3 per kg of body weight, amounted to about 60 per cent in comparison to the controls. The mechanism underlying this increase in pancreatic volume is discussed.

An *increase in the size of the islet tissue* was present in both groups, the differences being significant as compared to the values in the control groups. The increase in islet volume, expressed in mm^3 per kg of body weight, amounted to about 100 per cent in relation to the volume in the control groups.

The *alpha cell incidence* was somewhat lower in the biliary fistula animals than in the corresponding control group. In the biliary stasis animals, the alpha cell incidence was the same as in the controls. In all the groups, this incidence was within the normal range of variation.

TABLE 9

Statistical comparison between group means for size of total pancreas parenchyma and size of islet tissue in non-diabetic groups with functioning exocrine parenchyma

Groups	n	df	V_p	V_p/BW	n_i	n_i/BW	m_i	V_i	V_i/BW
I A —IV B	9(8)—9(8)	16(14)	*	***	***	***	*	***	***
II A —IV C	11—4	13	*	**	0	**	*	*	**
II A —IV E	11—4	13	*	*	**	**	0	**	**
IV C—IV E	4—4	6	0	0	*	0	0	0	0
II A —(IV C + IV E)	11—(4 + 4)	16	***	***	**	***	*	***	***
II A —IV B	11—9(8)	18(17)	***	***	**	***	**	***	***
I A —II A	9(8)—11	18(17)	0	0	0	0	0	*	0
(I A + II A)— (IV B + IV C + IV E)	[9(8) + 11]— [9(8) + 4 + 4]	32(30)	***	***	***	***	***	***	***

0 = no difference
* = probably significant difference
** = significant difference
*** = highly significant difference

TABLE 10

Body size and pancreas size in non-diabetic groups with functioning exocrine parenchyma

		I A	IV B	II A	IV C	IV E	IV D	IV A	IV F a
n		9	9	11	4	4	4	4	6
BW kg	$\bar{x}$	2.11	2.51	2.38	2.84	2.30	3.06	3.04	2.92
	$e(\bar{x})$	0.15	0.10	0.11	0.23	0.04	0.21	0.14	0.09
	s	0.45	0.31	0.36	0.46	0.07	0.41	0.27	0.21
BS dm^2	$\bar{x}$	15.8	17.0	16.7	18.0	16.4	18.6	18.6	18.2
	$e(\bar{x})$	0.51	0.31	0.35	0.64	0.12	0.55	0.36	0.23
	s	1.54	0.91	1.16	1.28	0.24	1.10	0.71	0.56
PW g	$\bar{x}$	4.77	3.60	6.89	4.47	3.05	5.05	4.31	3.79
	$e(\bar{x})$	0.49	0.20	0.51	0.44	0.23	0.69	0.35	0.27
	s	1.48	0.59	1.71	0.88	0.46	1.39	0.70	0.67
PW/BW g	$\bar{x}$	2.26	1.46	2.93	1.58	1.33	1.63	1.41	1.29
	$e(\bar{x})$	0.15	0.11	0.22	0.11	0.09	0.12	0.09	0.06
	s	0.45	0.32	0.72	0.23	0.19	0.24	0.17	0.16
V_p mm^3	$\bar{x}$	1,394.7	978.9	1,600.2	1,105.4	1,123.2	1,148.7	1,311.3	—
	$e(\bar{x})$	137.4	74.2	106.5	22.3	59.4	55.1	88.0	—
	s	412.1	222.5	353.4	44.5	118.8	110.1	176.0	—
V_p/BW mm^3	$\bar{x}$	671.6	396.8	685.9	399.0	489.7	377.5	432.5	—
	$e(\bar{x})$	61.5	32.7	52.9	40.0	26.4	16.3	29.5	—
	s	184.4	98.0	175.6	79.9	52.9	32.6	58.9	—

TABLE 11

Quantitative analysis of islet tissue in non-diabetic groups with functioning exocrine parenchyma

		I A	IV B	II A	IV C	IV E	IV D	IV A	IV F a
n		8	8	11	4	4	4	4	6
l	$\bar{x}$	10.3	8.7	13.5	11.5	8.3	11.8	13.5	13.3
k	$\bar{x}$	7.0	6.3	7.9	6.5	8.3	6.5	7.3	7.2
N	$\bar{x}$	601	594	232	193	207	189	221	291
n_i	$\bar{x}$	1,952.8	1,449.6	2,468.9	1,770.5	1,404.5	1,584.5	2,071.5	3,170.9
	$e(\bar{x})$	111.8	83.3	213.1	138.7	89.3	92.6	159.5	357.4
	s	316.2	235.5	706.7	277.3	178.6	185.1	318.9	875.3
n_i/BW	$\bar{x}$	959.1	592.6	1,046.2	627.0	610.8	522.3	683.3	1,089.7
	$e(\bar{x})$	84.0	51.8	84.7	33.4	31.0	38.0	55.4	127.7
	s	237.5	146.5	280.8	66.8	62.1	76.1	110.8	312.7
m_i μ^2	$\bar{x}$	3,530.4	3,088.6	3,709.8	2,950.8	3,214.8	3,598.5	3,706.5	3,739.7
	$e(\bar{x})$	164.1	120.1	156.7	371.3	200.4	304.8	297.0	333.2
	s	464.1	339.7	519.9	742.6	400.7	609.6	593.9	816.0
V_i mm^3	$\bar{x}$	8.26	5.41	11.04	6.34	5.39	6.86	9.06	14.89
	$e(\bar{x})$	0.57	0.46	1.15	1.03	0.36	0.72	0.38	2.88
	s	1.62	1.31	3.80	2.06	0.72	1.44	0.75	7.05
V_i/BW mm^3	$\bar{x}$	3.98	2.20	4.69	2.21	2.35	2.24	2.98	5.12
	$e(\bar{x})$	0.20	0.22	0.47	0.28	0.15	0.16	0.07	1.03
	s	0.58	0.61	1.55	0.56	0.29	0.33	0.13	2.53
$\alpha + \beta$	$\bar{x}$	1,400	1,320	1,446	1,403	1,359	1,654	1,622	1,437
α %	$\bar{x}$	13.51	21.34	16.76	15.66	19.54	18.85	20.10	16.14
	$e(\bar{x})$	1.66	1.71	1.92	2.02	3.40	1.18	1.94	2.52
	s	4.70	4.84	6.36	4.03	6.80	2.36	3.88	6.18

TABLE 12

Histochemical examination of the liver cells (approximative semi-quantitative grading). For explanation of grading, see page 49.

Group	Lipids	Glycogen	Alk. phosphatase
I A	0—1	3—4	3—4
II A	0—1	1—2	3—4
IV B	4—5	3—4	5—6
IV C	4—5	6—7	4—5
IV E	3	2—3	6
I B + II B	0—1	4—5	4—5
IV G	4—5	6—7	5—6
III A	4—5	6—7	6
IV A + IV D	5—6	6—7	6
III B	4—5	7	5—6
III C a	4—5	7	5—6
IV F a	4—5	6—7	6

CHAPTER 6

BILE FLOW EXPERIMENTS IN DIABETIC ANIMALS

Bile flow experiments were made on 11 alloxan-diabetic animals; 6 of them belonged to the biliary fistula *group I B,* and 5 to the biliary stasis *group II B.* In the former group, 5 animals (3 males and 2 females) were assigned to category 1, and 1 (male) to category 2. In the latter group (all males), 3 were assigned to category 1, and 2 to category 2. In addition, the effect of alloxan after ligation of the common bile duct was tested in 2 cases (both males): *group II C;* both belonged to category 2. These groups thus consisted of the following animals:

Group I B, category 1: R. 14, 193, 194, 216, 180
Group I B, category 2: R. 189
Group II B, category 1: R. 148, 149, 174
Group II B, category 2: R. 150, 175
Group II C, category 2: R. 231, 232.

Assignation to the respective categories was made on the same principles as described earlier. Thus, the three animals in groups I B and II B assigned to category 2 had either died less than two weeks after operation, or the post mortem changes were such that any quantitative micro-morphologic analysis was unfeasible. In group II C, I considered an analysis of this nature to be of little interest; this is why the animals were assigned to category 2.

The 19 alloxan-diabetic animals of *group IV G* served as controls. This group was divided into two sub-groups: *group IV G a,* in which a single diabetogenic alloxan dose of 200 mg/kg of body weight was given, and *group IV G b,* in which the dose of alloxan was 100 mg/kg of body weight on two successive days. Group IV G a comprised 11 animals; 4 (3 males and 1 female) were assigned to category 1, and 7 (6 males and 1 female) to category 2. Group IV G b consisted of 8 animals; 5 (2 males and 3 females) belonged to category 1, and 3 (1 male and 2 females) to category 2. Here

as well, the length of the observation period and the occurrence of post mortem changes determined the category to which the animal was assigned. Group IV G thus consisted of the following animals:

Group IV G a, category 1: R. 143, 146, 147, 165
Group IV G a, category 2: R. 141, 151, 181, 187, 188, 190, 164
Group IV G b, category 1: R. 243, 245, 246, 247, 248
Group IV G b, category 2: R. 203, 204, 244.

In view of the relatively small number of animals in groups I B and II B, as well as of the results of bile flow experiments on non-diabetic animals, which showed the same effect on the pancreas in both biliary fistula and biliary stasis (Chapter 5), the results in groups I B and II B will be accounted for together. The salient features of the experiments and of the post mortem observations are given in the following animal records.

ANIMAL RECORDS

GROUP I B: CATEGORY 1

R. 14. Female. 1st obs. day 27/2 1952. BW 1.85 kg.—29/2 BW 1.91 kg. *Alloxan:* 200 mg/kg = 380 mg.—3/3 Massive glycosuria, about 8 %. Hyperglycaemia, blood sugar 446 mg/100 ml. Condition good. PZI 8 u/24 hrs started.—7/3 Insulin increased to 12 u/24 hrs; hypoglycaemic convulsions in p. m.; stopped by glucose.—13/3 Diabetes unchanged, condition good. No acetonuria.—14/3 Operation: *Biliary fistula* (glass cannula). BW 1.93 kg. Anaesthetic: 63 mg kemithal.—16/3 Hypoglycaemic convulsions in p. m.; stopped by glucose. Insulin temporarily discontinued. Copious bile flow.—20/3 Hyperglycaemia persists, blood sugar 418 mg/100 ml. Insulin restarted: 4 u PZI/24 hrs.—23/3 Blood sugar normal; no glycosuria. Insulin definitely discontinued. Copious bile flow. Condition good.—3/4 For past 10 days blood sugar consistently normal; no glycosuria. Copious bile flow. BW 1.40 kg. Killed. *Autopsy:* extensive abdominal adhesions around fistula tube. Fairly great reduction in subcutaneous and intra-abdominal adipose tissue. On liver serosa, moderate number of white spots, size of a pin's head, partly confluent. Cut surface of liver: N.A.D. Pancreas: N.A.D. (not weighed).—*Micr. exam.* of pancreas performed somewhat differently to that in rest of material (see p. 152). For course in this case, see also Fig. 37.

R. 193. Male. 1st obs. day 18/5 1954. BW 2.93 kg.—20/5 BW 3.05 kg. *Alloxan:* 100 mg/kg = 305 mg.—21/5 *Alloxan:* same dose as 20/5.—22/5 Massive glycosuria; insulin started.—30/5 With 22 u insulin/24 hrs (10 regular + 12 NPH) glycosuria about 10 g/24 hrs; no acetonuria, condition good.—31/5 Operation: *Biliary fistula* (polyethylene tubing). BW 3.05 kg. Anaesthetic: 100 mg kemithal. On this day, insulin divided into 2 doses; 20 ml of 30 % glucose subcutaneously at same time.—2/6 Condition fairly good but eats little. Copious bile flow. Only slight glycosuria.—6/6 Livelier, eats and drinks. Copious bile flow. Insulin: 4 u NPH/24 hrs; glycosuria less than 10 g.—14/6 Hypoglycaemic convulsions in p. m., stopped by glucose. Insulin definitely discontinued—21/6 Glycosuria about 1—5 g/24 hrs. Blood sugar 268 mg/100 ml. Copious bile flow. Condition good.—28/6 Condition unchanged, but jaundiced for past few days. Bilirubin test on urine weakly positive. Blood sugar 240 mg/100 ml. BW 2.83 kg. Killed. *Autopsy:* no adipose

tissue visible subcutaneously, in omentum or perirenally. Extensive adhesions in superior part of abdominal cavity. In left lateral lobe of liver, well defined abscess cavity about 30 mm in diameter. Occasional white spots, size of a pin's head, on cut surface of liver; otherwise N.A.D. Pancreas: N.A.D. PW 6.55 g (D 4.60 g, L 1.95 g).—*Micr. exam.:* increased perilobular connective tissue in liver and a few necrotic foci.

R. 194. Male. 1st obs. day 18/5 1954. BW 2.39 kg.—20/5 BW 2.46 kg. *Alloxan:* 100 mg/kg = 245 mg.—21/5 *Alloxan:* same dose as 20/5.—22/5 Massive glycosuria; insulin started.—1/6 Insulin: 10 u/24 hrs (4 regular + 6 NPH); glycosuria about 5 g/24 hrs; condition good, no acetonuria.—3/6 Operation: *Biliary fistula* (polyethylene tubing). BW 2.57 kg. Anaesthetic: ether + 100 mg kemithal. On this day, insulin divided into 2 doses; 20 ml of 30 % glucose subcutaneously at same time.—5/6 Condition fairly good. Only slight glycosuria. 4 u NPH insulin. Copious bile flow.—6/6 Glycosuria less than 1 g/24 hrs. Insulin discontinued.—9/6 Still scarcely measurable glycosuria. Blood sugar 125 mg/100 ml. Condition good. Copious bile flow.—13/6 Condition unchanged. Blood sugar 91 mg/100 ml.—15/6 No bile in bag.—18/6 Still no bile flow but condition good. Slight increase in glycosuria to about 4 g/24 hrs. No acetonuria. No insulin given.—30/6 For past 12 days, average excretion of glucose 4.2 g/24 hrs. No acetonuria. No proteinuria. No bile flow since 14/6. No insulin given. Condition good despite marked jaundice. Bilirubin test on urine strongly positive. In p. m. blood sugar 174 mg/100 ml. BW 2.35 kg. Killed. *Autopsy:* no adipose tissue visible subcutaneously, in omentum or perirenally. Tissues generally icteric. Inappreciable abdominal adhesions. Cessation of bile flow explained by occlusion of polyethylene tubing close to gallbladder by soft gallstones. Liver cirrhotic, firm and tough. No foci or spots on its serosa or cut surface. Pancreas: N.A.D., no fat. PW 5.26 g (D 3.38 g, L 1.88 g).—*Micr. exam.:* plentiful perilobular connective tissue in liver, entirely separating lobules—biliary cirrhosis. Fairly extensive fatty degeneration of cirrhotic connective tissue.

R. 216. Female. 1st obs. day 1/10 1954. BW 2.48 kg.—4/10 BW 2.51 kg. *Alloxan:* 100 mg/kg = 250 mg.—5/10 *Alloxan:* same dose as 4/10.—8/10 Hitherto no glycosuria. BW 2.53 kg. *Alloxan:* 200 mg/kg = 505 mg.—15/10 Still no glycosuria. BW 2.42 kg. *Alloxan:* 300 mg/kg = 725 mg.—17/10 Massive glycosuria. Insulin started.—17/11 With 8 u insulin/24 hrs (4 regular + 4 NPH) glycosuria about 10 g/24 hrs. Condition good. No acetonuria. BW 2.55 kg. Operation: *Biliary fistula* (polyethylene tubing). Anaesthetic: ether.—19/11 Condition slightly affected. Glycosuria 3 g/24 hrs. No insulin. Fairly copious bile flow.—24/11 Condition improved. Slightly increased glycosuria. Insulin restarted: 4 u/24 hrs. Continued bile flow.—29/11 No bile in bag. Condition good. Excretion of glucose about 10 g/24 hrs. Insulin: 6 u/24 hrs.—15/12 Condition and appetite still good. No bile flow since 29/11; jaundice. Bilirubin test on urine strongly positive. For past 7 days, insulin: 4 u/24 hrs; average excretion of glucose 5 g/24 hrs. BW 2.41 kg. Killed. *Autopsy:* marked generalized jaundice. Considerable but not total reduction in adipose tissue. Considerable connective tissue formation around fistula tube; lumen occluded by soft green concretions. Liver firm, tough, cirrhotic. Pancreas: N.A.D. PW 4.49 g (D 2.79 g, L 1.70 g).—*Micr. exam.:* considerable perilobular increase in connective tissue of liver. Bile ducts dilated.

R. 180. Male. 1st obs. day 26/3 1954. BW 2.72 kg.—29/4 Glucose tolerance test. BW 3.06 kg. Glucose: 4.9 ml.—17/5 BW 3.29 kg. *Alloxan:* 200 mg/kg = 660 mg.—19/5 Massive glycosuria, acetonuria and proteinuria. Insulin started.—31/5 With 32 u insulin/24 hrs (16 regular + 16 NPH) excretion of glucose about 15 g/24 hrs. Condition good. No acetonuria.—1/6 Operation: *Biliary fistula* (polyethylene tubing). BW 2.89 kg. Anaesthetic: ether + 100 mg kemithal. On this day, insulin divided into 2 doses; 20 ml of 30 % glucose subcutaneously at same time.—3/6 Condition good. Copious bile flow. Moderate glycosuria. Insulin 6 u.—14/6 Condition still good. Bile flow about 100 ml/24 hrs. Glycosuria about 10 g/24 hrs. Blood sugar 298 mg/100 ml. Insulin: 12 u NPH/24 hrs.—29/6 Condition consistently very good, appetite normal. Copious bile flow; thus

in past 24 hrs 112 ml. No jaundice. Bilirubin test on urine negative. Glycosuria about 10 g/24 hrs. No acetonuria. Insulin: 10 u NPH. Blood sugar 308 mg/100 ml. BW 3.04 kg. Killed. *Autopsy:* normal amount of adipose tissue. No icteric discoloration of tissues. Connective tissue capsule around fistula tube. Liver and pancreas: N.A.D. PW 4.25 g (D. 2.40 g, L 1.85 g).—*Micr. exam.:* slight increase in connective tissue of liver perilobularly, otherwise N.A.D.

GROUP I B: CATEGORY 2

R. 189. Male. 1st obs. day 5/5 1954. BW 3.05 kg.—12/5 BW 2.92 kg. *Alloxan:* 200 mg/kg = 585 mg.—16/5 Massive glycosuria, acetonuria and proteinuria. Insulin started.—27/5 Insulin: about 32 u/24 hrs (16 regular + 16 NPH); glycosuria about 20 g/24 hrs. No acetonuria. Condition good.—28/5 Operation: *Biliary fistula* (polyethylene tubing). BW 2.81 kg. Anaesthetic: ether + 100 mg kemithal.—30/5 Condition poor; did not eat. Acetonuria. Insulin: 20 u.—31/5 Some improvement but still acetonuria. Insulin: 24 u. Moderate bile flow.—2/6 Livelier, started eating. No acetonuria. Insulin: 12 u. In p. m. hypoglycaemic convulsions; stopped by glucose.—3/6 Fairly lively. No acetonuria. Bile flow continuous. No insulin.—4/6 Considerably worse; massive acetonuria. Died during day. *Autopsy:* BW 2.38 kg. No reduction in adipose tissue. Extensive loose adhesions around fistula tube. A few punctate white spots on liver surface beside gall-bladder. Liver otherwise N.A.D. Pancreas: N.A.D. Probable cause of death: diabetic acidosis.—*Micr. exam.:* nothing definitely pathologic in liver.

GROUP II B: CATEGORY 1

R. 148. Male. 1st obs. day 29/12 1953. BW 3.52 kg.—12/1 BW 3.24 kg. *Alloxan:* 200 mg/kg = 650 mg.—14/1 Massive glycosuria; insulin started.—1/2 With 12 u NPH insulin/24 hrs, glycosuria about 3 %, corresponding to about 10 g of glucose/24 hrs. No acidosis. Condition good.—2/2 Operation: *Ligation of common bile duct.* BW 3.46 kg. Anaesthetic: ether.—4/2 Condition fairly good. Glycosuria 3.1 g/24 hrs. Insulin: 4 u NPH/24 hrs.—14/2 Condition unchanged. Glycosuria about 10 g/24 hrs. Insulin: 6 u NPH/24 hrs.—1/3 Condition unchanged. Glycosuria about 5 g/24 hrs. Insulin: 4 u NPH/24 hrs.—9/3 Consistently good condition and appetite. Jaundice. Diabetes unchanged during past week; glycosuria about 10 g/24 hrs, insulin: 4 u NPH/24 hrs. No acetonuria or proteinuria. Bilirubin test on urine strongly positive. BW 3.22 kg. Killed. *Autopsy:* generalized jaundice. Total lack of adipose tissue. About 100 ml of yellow opalescent fluid in abdominal cavity. Liver considerably enlarged and firm; granulated surface. Bile ducts dilated. Otherwise N.A.D. PW 4.63 g (D 3.06 g, L 1.57 g).—*Micr. exam.:* liver, biliary cirrhosis; moderate fatty degeneration of cirrhotic connective tissue. No fat in liver cells.

R. 149. Male. 1st obs. day 29/12 1953. BW 3.48 kg.—15/1 BW 3.26 kg. *Alloxan:* 200 mg/kg = 650 mg.—18/1 Massive glycosuria, acetonuria and proteinuria. Condition somewhat affected; lethargic. Insulin started.—20/1 In p. m., hypoglycaemic convulsions; stopped by glucose.—27/1 Condition good. Glycosuria about 6 g/24 hrs; insulin: 8 u NPH/24 hrs. No acetonuria.—28/1 Operation: *Ligation of common bile duct.* BW 3.17 kg. Anaesthetic: ether.—30/1 Condition fairly good. Moderate glycosuria. No acidosis. Insulin: 6 u NPH/24 hrs.—10/2 Condition and appetite good. Glycosuria about 3 %, corresponding to about 8 g/24 hrs. No acetonuria or proteinuria. Insulin: 6 u/24 hrs.—20/2 Condition unchanged. Glycosuria 5 g/24 hrs. Insulin: 2 u/24 hrs. No acetonuria.—4/3 Condition and appetite consistently good. Past 24 hrs glycosuria about 3 g; no acetonuria. Insulin: 2 u NPH/24 hrs. Bilirubin test on urine strongly positive. BW 3.15 kg. Killed. *Autopsy:* generalized jaundice. Total lack of adipose tissue. About 100 ml of clear yellowish-brown fluid in abdominal cavity. Liver enlarged, greenish-brown, firm and tough. Bile ducts dilated. Considerable enlargement of spleen. Otherwise N.A.D. PW 5.86 g (D 3.57 g, L 2.29 g).—*Micr. exam.:* liver, severe biliary cirrhosis.

R. 174. Male. 1st obs. day 2/3 1954. BW 2.98 kg.—15/3 BW 3.07 kg. *Alloxan:* 200 mg/kg = 615 mg.—17/3 Massive glycosuria, proteinuria. No acetonuria. Insulin started.—18/3 Condition affected, lethargic, forced respiration. Acetonuria. 32 u regular insulin in 3 doses, as well as bicarbonate and glucose solution subcutaneously.—19/3 Condition still greatly affected and heavy acetonuria. Same treatment as previous day. At 1 p. m., blood sugar 630 mg/100 ml.—20/3 Livelier, starts to eat. No acetonuria in p. m.—22/3 Condition good. During past 24 hrs, glycosuria 10.5 g. No acetonuria or proteinuria. Insulin: 22 u (8 regular + 14 NPH).—31/3 During past week, condition good despite glycosuria over 20 g/24 hrs. Insulin: 30 u/24 hrs (12 regular + 18 NPH). No acetonuria.—1/4 Operation: *Ligation of common bile duct*. BW 2.94 kg. Anaesthetic: ether.—3/4 Condition fairly good, eats a little.—10/4 Condition good. During past 24 hrs, glycosuria 7.1 g. Insulin: 14 u (6 regular + 8 NPH).—20/4 Condition still good, appetite normal. During past 24 hrs, glycosuria 6.7 g. Insulin: 10 u (4 regular + 6 NPH).—28/4 Condition unchanged. Glycosuria 5—10 g/24 hrs during past few days. No acetonuria. For past week, insulin 4 u NPH/24 hrs. No proteinuria. BW 2.83 kg. Killed. *Autopsy:* generalized jaundice. Almost complete lack of adipose tissue. About 5 ml of clear yellow fluid in abdominal cavity. Liver enlarged, yellowish-brown, firm and tough. Bile ducts dilated. PW 5.53 g (D 4.06 g, L 1.47 g).—*Micr. exam.*: liver, biliary cirrhosis; moderate fatty degeneration of cirrhotic connective tissue. No fat in liver cells.

GROUP II B: CATEGORY 2

R. 150. Male. 1st obs. day 29/12 1953. BW 3.48 kg.—12/1 BW 3.24 kg. *Alloxan:* 200 mg/kg = 650 mg.—14/1 Massive glycosuria. No acetonuria. Insulin started.—25/1 Condition good. Glycosuria about 10—20 g with 12 u NPH insulin/24 hrs. No acetonuria or proteinuria.—26/1 Operation: *Ligation of common bile duct*. BW 3.34 kg. Anaesthetic: ether.—27/1 Condition poor; lethargic, no appetite.—28/1 Found dead in morning. *Autopsy:* BW 3.19 kg. Large quantities of foetid, reddish-brown, fibrin-mixed pus in abdominal cavity. Duodenum perforated and partly gangrenous.—No *micr. exam.* made.

R. 175. Male. 1st obs. day 2/3 1954. BW 3.06 kg.—15/3 BW 3.07 kg. *Alloxan:* 200 mg/kg = 615 mg.—17/3 Glycosuria, acetonuria, proteinuria. Condition fairly good. Insulin started.—18/3 Condition affected; lethargic, forced respiration. Massive glycosuria and acetonuria. 32 u regular insulin in 3 doses, as well as bicarbonate and glucose solution subcutaneously.—19/3 Condition unchanged. Still heavy glycosuria and acetonuria. At 1 p. m., blood sugar 568 mg/100 ml. 46 u insulin in 3 doses, as well as bicarbonate and glucose subcutaneously.—20/3 Condition improved; started to eat. Still acetonuria.—23/3 No acetonuria. Much livelier. Insulin: 22 u (8 regular + 14 NPH) in single dose.—29/3 Condition good. Glycosuria 10—20 g/24 hrs. Insulin: 22 u (12 regular + 10 NPH).—30/3 Operation: *Ligation of common bile duct*. BW 2.67 kg. Anaesthetic: ether.—1/4 Condition somewhat affected, appetite impaired. Oliguria. Inappreciable glycosuria. No acetonuria. 6 u regular insulin.—3/4 Livelier. Glycosuria 3.3 g/24 hrs. Insulin: 10 u.—6/4 In p. m. hypoglycaemic symptoms; stopped by glucose.—8/4 Oliguria. Glycosuria less than 1 g/24 hrs. Insulin discontinued. No acetonuria.—11/4 During past 24 hrs, glycosuria 8.0 g. 4 u NPH insulin.—18/4 For past week, glycosuria about 2 g/24 hrs. No acetonuria or proteinuria. 4 u regular insulin/24 hrs. Condition consistently affected, appetite poor. Found dead in morning. *Autopsy:* BW 2.17 kg. Purulent keratitis in right eye. Generalized jaundice. Total lack of adipose tissue. Fairly plentiful foetid, turbid fluid in abdominal cavity. In superior part of abdomen, adhesions between liver, duodenum and omentum. Fibrinous deposits on peritoneum of whole abdomen. Perforation of bile ducts into omentum, in which bile was collected in thin-walled, saccular dilatations. Greenish discoloration of liver; on cut surface, almost totally necrotic. Pancreas could not be dissected free from surrounding tissues.—*Micr. exam.:* liver, numerous necrotic foci; relatively rich in fat.

GROUP IV G a: CATEGORY 1

R. 143. Male. 1st obs. day 13/10 1953.—17/10 BW 2.21 kg.—19/10 Glucose tolerance test. BW 2.04 kg. Glucose: 3.3 ml.—20/11 BW 2.43 kg. *Alloxan:* 200 mg/kg = 485 mg.—22/11 Massive glycosuria. Insulin started.—6/1 Continuous glycosuria since 22/11; average 11.3 g/24 hrs. Insulin: 12—14 u NPH/24 hrs. Condition good. BW 2.49 kg. Killed. *Autopsy:* N.A.D. PW 3.69 g (D. 2.00 g, L 1.69 g).

R. 146. Male. 1st obs. day 3/11 1953.—6/11 Glucose tolerance test. BW 2.71 kg. Glucose: 4.3 ml.—12/11 BW 2.95 kg. *Alloxan:* 200 mg/kg = 590 mg.—14/11 Massive glycosuria. No acetonuria. Insulin started.—28/12 Continuous glycosuria since 14/11; average 8.5 g/24 hrs. Insulin: 4—8 u NPH/24 hrs. Condition good. BW 3.38 kg. Killed. *Autopsy:* N.A.D. PW 5.21 g (D 3.91 g, L 1.30 g).

R. 147. Male. 1st obs. day 3/11 1953.—6/11 Glucose tolerance test. BW 2.44 kg. Glucose: 3.9 ml.—16/11 BW 2.73 kg. *Alloxan:* 200 mg/kg = 545 mg.—18/11 Massive glycosuria. Insulin started.—28/12 Continuous glycosuria since 18/11; about 10.9 g/24 hrs. Insulin: 8—12 u NPH/24 hrs. Condition good. BW 3.07 kg. Killed. *Autopsy:* N.A.D. PW 4.50 g (D 2.35 g, L 2.15 g).

R. 165. Female. 1st obs. day 2/2 1954.—3/2 BW 2.84 kg.—20/2 BW 2.91 kg. *Alloxan:* 200 mg/kg = 580 mg.—22/2 Massive glycosuria and proteinuria. Acetonuria. Insulin started. Worse during day; lethargic, did not eat. Acetone in urine strongly positive. 16 u insulin (12 regular + 4 NPH) as well as bicarbonate and glucose solution subcutaneously.— 23/2 Livelier, started to eat.—25/2 Still fairly lethargic; ate little. Still acetonuria. Treatment as earlier.—28/2 Condition unchanged.—3/3 Considerable improvement. No acetonuria for first time since 22/2.—1/4 Condition good. Glycosuria about 20 g/24 hrs. Insulin: 24 u/24 hrs (8 regular + 16 NPH).—27/4 Glycosuria unchanged. No acetonuria or proteinuria. Insulin 18 u (6 regular + 12 NPH). Condition good. BW 3.62 kg. Killed. *Autopsy:* N.A.D. PW 5.21 g (D 3.00 g, L 2.21 g).

GROUP IV G a: CATEGORY 2

R. 141. Male. 1st obs. day 13/10 1953.—15/10 Glucose tolerance test. BW 2.06 kg. Glucose: 3.3 ml.—18/11 BW 2.83 kg. *Alloxan:* 200 mg/kg = 565 mg.—1/12 Hitherto no glycosuria. BW 2.61 kg. *Alloxan:* 200 mg/kg = 520 mg.—3/12 Massive glycosuria. No acetonuria. Insulin started.—10/12 Found dead in morning. *Autopsy:* BW 2.68 kg. Internal organs: N.A.D. Cause of death could not be definitely established (hypoglycaemia?). PW 4.29 g (D 2.95 g, L 1.34 g).

R. 151. Male. 1st obs. day 29/12 1953.—31/12 BW 3.48 kg.—15/1 BW 3.51 kg. *Alloxan:* 200 mg/kg = 700 mg.—16/1 Glycosuria, proteinuria. No acetonuria.—17/1 Condition worse; lethargic, no appetite. Protein in urine strongly positive. No acetonuria. Died during day. *Autopsy:* BW 3.33 kg. Lipaemia. Liver swollen; otherwise N.A.D. Kidneys enlarged; cut surface swollen. Adrenals enlarged; haemorrhages in cortex. Pancreas difficult to dissect out, owing to hyperaemia of surrounding tissues. D part could therefore not be obtained free from fat to usual extent. PW 9.08 g (D 7.49 g, L 1.59 g).—*Micr. exam.:* a few perilobular necrotic foci in liver. Severe fatty degeneration. Kidneys: extensive tubular necrosis. Adrenals: necrotic foci with haemorrhages.—Cause of death: toxic parenchymal damage.

R. 181. Male. 1st obs. day 26/3 1954. BW 2.35 kg.—29/4 Glucose tolerance test. BW 2.46 kg. Glucose: 3.9 ml.—17/5 BW 2.82 kg. *Alloxan:* 200 mg/kg = 565 mg. Condition greatly affected in connexion with injection; air hunger and cyanosis. Died 3 hrs later. *Autopsy:* Pulmonary oedema. Otherwise N.A.D.—No *micr. exam.* made.

R. 187. Male. 1st obs. day 5/5 1954. BW 2.84 kg.—10/5 BW 2.55 kg. *Alloxan:* 200 mg/kg = 510 mg.—12/5 Glycosuria and proteinuria. Slight acetonuria.—13/5 Condition successively worse during day. Heavy acetonuria. Insulin therapy and parenteral fluids ineffective. Died. *Autopsy:* BW 2.30 kg. Lipaemia. Liver enlarged; cut surface swollen. Kidneys enlarged; cut surface

granulated. Adrenals enlarged. Otherwise N.A.D. PW 4.03 g (D 2.99 g, L 1.04 g).—*Micr. exam.*: circumscribed necrotic areas in liver and fairly marked fatty degeneration of cells. Kidneys: tubular necrosis. Adrenals: a few necrotic areas. Pancreas (no systematic quantitative analysis of islets): small islets with numerous alpha cells; scattered remains of necrotic beta cells.

R. 188. Male. 1st obs. day 5/5 1954. BW 3.00 kg.—10/5 BW 2.78 kg. *Alloxan:* 200 mg/kg = 555 mg.—12/5 Slight glycosuria; proteinuria. No acetonuria.—13/5 Increased glycosuria; in p. m. acetonuria as well. Condition poor. Insulin started.—14/5 Condition unchanged. Insulin therapy and parenteral fluids ineffective. Died. *Autopsy:* BW 2.73 kg. Oedema of abdominal wall. Liver and kidneys enlarged; cut surface swollen and granulated. Cause of death: toxic parenchymal damage.—No *micr. exam.* made.

R. 190. Male. 1st obs. day 5/5 1954. BW 2.64 kg.—12/5 BW 2.67 kg. *Alloxan:* 200 mg/kg = 535 mg.—14/4 Massive glycosuria, proteinuria and acetonuria. Insulin started.—17/5 Insulin therapy and parenteral fluids ineffective. Died during day. *Autopsy:* Lipaemia. Liver enlarged; yellowish-brown, granulated. Kidneys enlarged; cut surface granulated. Adrenals and pancreas N.A.D.—*Micr. exam.:* extensive liver necrosis and fatty degeneration. Kidneys: widespread tubular necrosis.

R. 164. Female. 1st obs. day 2/2 1954. BW 3.36 kg.—20/2 BW 3.33 kg. *Alloxan:* 200 mg/kg = 665 mg.—21/2 Inappreciable glycosuria, heavy proteinuria. No acetonuria.—22/2 Considerably worse; forced respiration, massive glycosuria and acetonuria. Died. *Autopsy:* BW 3.22 kg. Lipaemia. Liver and kidneys enlarged with swollen cut surface. Pancreas: N.A.D. PW 4.86 g (D 2.62 g, L 2.24 g).—*Micr. exam.:* perilobular cellular infiltration in liver and incipient necrosis. Kidneys: fairly extensive tubular necrosis.

GROUP IV G b: CATEGORY 1

R. 243. Male. 1st obs. day 25/1 1955. BW 3.39 kg.—8/2 BW 3.27 kg. *Alloxan:* 100 mg/kg = 330 mg.—9/2 *Alloxan:* same dose as 8/2.—10/2 Massive glycosuria. No acetonuria. Insulin started.—10/3 Continuous glycosuria since 10/2; average 21.4 g/24 hrs. Insulin: average 20 u/24 hrs; past week 22 u (10 regular + 12 NPH). Condition good. BW 3.52 kg. Killed. *Autopsy:* N.A.D. PW 3.35 g (D 2.26 g, L 1.09 g).

R. 245. Male. 1st obs. day 25/1 1955. BW 2.29 kg.—8/2 BW 2.27 kg. *Alloxan:* 100 mg/kg = 230 mg.—9/2 *Alloxan:* same dose as 8/2.—10/2 Massive glycosuria. No acetonuria. Insulin started.—10/3 Continuous glycosuria since 10/2; average 16.6 g/24 hrs. Insulin: average 18 u/24 hrs; past 2 weeks 20 u (10 regular + 10 NPH). Condition good. BW 2.64 kg. Killed. *Autopsy:* All adipose tissue yellow. Otherwise N.A.D. PW 2.68 g (D 1.67 g, L 1.01 g).

R. 246. Female. 1st obs. day 25/1 1955. BW 3.93 kg.—15/2 BW 4.03 kg. *Alloxan:* 100 mg/kg = 405 mg.—16/2 *Alloxan:* same dose as 15/2.—17/2 Massive glycosuria. No acetonuria. Insulin started.—16/3 Continuous glycosuria since 17/2; average 25 g/24 hrs. Insulin: average 18 u/24 hrs; past week 24 u (12 regular + 12 NPH). Condition good. BW 4.00 kg. Killed. *Autopsy:* several scarred pits in kidneys. Otherwise N.A.D. PW 3.89 g (D 2.51 g, L 1.38 g).—*Micr. exam.:* wide streaks of highly cellular connective tissue in kidneys, corresponding to macroscopic changes; parenchyma otherwise normal.

R. 247. Female. 1st obs. day 25/1 1955. BW 2.92 kg.—15/2 BW 3.30 kg. *Alloxan:* 100 mg/kg = 330 mg.—16/2 *Alloxan:* same dose as 15/2.—17/2 Massive glycosuria. No acetonuria. Insulin started.—17/3 Continuous glycosuria since 17/2; average 11.4 g/24 hrs. Insulin: average 8 u/24 hrs; past few days 6 u/24 hrs (2 regular + 4 NPH). Condition good. BW 3.88 kg. Killed. *Autopsy:* N.A.D. PW 5.18 g (D 3.33 g, L 1.85 g).

R. 248. Female. 1st obs. day 25/1 1955. BW 3.01 kg.—15/2 BW 2.96 kg. *Alloxan:* 100 mg/kg = 295 mg.—16/2 *Alloxan:* same dose as 15/2.—17/2 Massive glycosuria. No acetonuria. Insulin

started.—17/3 Continuous glycosuria since 17/2; average 16.0 g/24 hrs. Insulin: average 16 u/24 hrs; past few days 18 u/24 hrs (8 regular + 10 NPH). Condition good. BW 3.33 kg. Killed. *Autopsy*: NA.D. PW 3.64 g (D 2.53 g, L 1.11 g).

GROUP IV G b: CATEGORY 2

R. 203. Female. 1st obs. day 23/7 1954. BW 2.05 kg.—28/7 BW 2.07 kg. *Alloxan:* 100 mg/kg = 205 mg.—29/7 *Alloxan:* same dose as 28/7.—30/7 Glycosuria 2.5 g/24 hrs. No acetonuria.—31/7 Glycosuria less than 1 g/24 hrs.—6/8 Glycosuria scarcely measurable for past few days (within normal borderlines). BW 2.23 kg. *Alloxan:* 100 mg/kg = 225 mg.—9/8 Still scarcely measurable glycosuria. BW 2.32 kg. *Alloxan:* 150 mg/kg = 350 mg.—10/8 Glycosuria 12 g.—11/8 Glycosuria 2.3 g.—13/8 Glycosuria 0.2 g.—15/8 Glycosuria 9.6 g.—16/8 Glycosuria 11.5 g.—17/8 Glycosuria 1.7 g.—18/8 Glycosuria 0.2 g.—20/8 Found dead; no previous signs of illness. Glycosuria not constant, despite repeated doses of alloxan. *Autopsy:* BW 2.42 kg. Somewhat increased fluid in abdominal cavity. Scanty fibrin deposits on intestines. Otherwise N.A.D. Cause of death could not be definitely established. —Owing to post mortem changes, no *micr. exam.* made.

R. 204. Female. 1st obs. day 23/7 1954. BW 1.98 kg.—28/7 BW 2.04 kg. *Alloxan:* 100 mg/kg = 205 mg.—29/7 *Alloxan:* same dose as 28/7.—30/7 Glycosuria.—5/8 Continuous glycosuria since 30/7, although relatively small 24-hr excretion. For past few days 4 u NPH insulin/24 hrs. Blood sugar (11 a. m.) 321 mg/100 ml.—6/8 Acute exacerbation; lethargic, no appetite. No signs of acidosis. Died. *Autopsy:* N.A.D. Cause of death could not be definitely established.

R. 244. Male. 1st obs. day 25/1 1955. BW 2.87 kg.—8/2 BW 3.05 kg. *Alloxan:* 100 mg/kg = 305 mg.—9/2 *Alloxan:* same dose as 8/2.—10/2 Massive glycosuria. No acetonuria. Insulin started.—15/2 Still considerable glycosuria. Insulin: 16 u/24 hrs (8 regular + 8 NPH). In p. m. condition affected; unable to use hind legs. Glucose administration ineffective.—19/2 Somewhat livelier but hinds legs paralyzed. Glycosuria unchanged.—21/2 Convulsions in p. m. No immediate effect of glucose. Moribund; therefore killed. BW 2.98 kg. *Autopsy:* N.A.D. Cause of symptoms could not be established. PW 3.50 g (D 2.20 g, L 1.30 g).—*Micr. exam.:* liver, kidneys and adrenals N.A.D.

ALLOXAN ADMINISTRATION

In *group I B* (6 animals), three (R. 14, 180 and 189) were given a single dose of 200 mg/kg of body weight, and the other three (R. 193, 194 and 216) a dose of 100 mg/kg on two successive days. With the exception of R. 216, these animals became diabetic 24 to 48 hours after administration of alloxan. R. 216 did not react to the two 100 mg/kg doses, nor to a somewhat later dose of 200 mg/kg. When, however, the dose was increased to 300 mg/kg, massive and continuous glycosuria appeared. In R. 180 and R. 189, the initial stage of diabetes was associated with acetonuria for 4 and 3 days, respectively, and proteinuria for 3 and 5 days, respectively. The general condition was unaffected and the symptoms yielded to insulin therapy. Neither acetonuria nor proteinuria was present in the other animals.

In *group II B* (5 animals), all were given a single dose of 200 mg/kg, which resulted in the appearance of diabetes in every case. In three of

them (R. 149, 174 and 175) massive acetonuria was initially present for 3, 3 and 7 days, respectively, and proteinuria for 4, 2 and 2 days, respectively. All exhibited generalized symptoms of acidosis, lethargy and forced respiration. Coma was inhibited and the general condition normalized by means of relatively large doses of regular insulin, and parenteral administration of bicarbonate and glucose solutions.

In control *group IV G a* (11 animals) nine animals became diabetic after administration of alloxan. One (R. 141) did not react, but after a fresh dose of the same size was given 13 days later, massive glycosuria appeared. One animal (R. 181) died of pulmonary oedema in direct association with injection of alloxan; it was therefore impossible to judge the effect in this case. An additional five animals (R. 151, 187, 188, 190 and 164) died within 5 days of alloxan administration. Death was caused by toxic alloxan damage to the liver and kidneys and diabetic acidosis. Energetic insulin and fluid therapy were thus unable to save these animals. Microscopic examination showed circumscribed necrotic areas in the liver, and more or less extensive necrosis of the renal tubules. In two cases (R. 151 and 187) necrotic changes and haemorrhages were also present in the adrenal cortex. One animal (R. 141) died overnight, 9 days after administration of alloxan, without any previous indications of illness in addition to diabetes. Autopsy failed to disclose the cause of death, although it may have been hypoglycaemia resulting from insulin overdosage (18 u the day before death).

Of the remaining four animals, R. 165 had heavy acidosis in connexion with the onset of diabetes; it did not disappear until after 8 days' intensive insulin and fluid therapy. The subsequent course was uneventful.

In control *group IV G b* (8 animals), seven became diabetic in direct association with alloxan administration. The remaining animal (R. 203) had slight transient glycosuria, which increased only inappreciably after a renewed dose of alloxan. Death occurred before the diabetogenic effect could be definitely evaluated. The cause of death could not be ascertained at autopsy. One animal (R. 204) died 9 days after the first injection of alloxan; in this case as well, the cause of death could not be established. In R. 244, paralysis of the hind legs appeared one week after the first injection; the animal died in convulsions 6 days later. No definite cause of death could be found; the diabetes had been under satisfactory control the whole time.

Acetonuria appeared in none of the animals in this group, but proteinuria of one to four days' duration was present in every case except R. 246.

To sum up, alloxan was administered to 35 previously untreated, healthy animals, *i. e.*, the 30 listed in the aforegoing, and 5 animals in group III B

(see Chapter 7). Of these 35 animals, 19 received a single dose of 200 mg/kg of body weight, and 16 a dose of 100 mg/kg on two successive days. The diabetogenic effect is recorded in Table 22, which shows that 17 and 14 animals, respectively, became diabetic at once, an additional 2 became diabetic after renewed injection of alloxan, and in the remaining 2 cases the effect could not be evaluated owing to death of the animal. Thus, no definite resistance to alloxan was noted in any of the cases.

PRE- AND POSTOPERATIVE COURSE

General Comments

After the diagnosis of diabetes had been established and the condition regulated by insulin therapy, the bile flow interventions were performed in the same way as in groups I A and II A. The preoperative duration of diabetes was as follows: group I B, category 1: mean, 17.4 days (14, 11, 14, 33 and 15 days); R. 189 (group I B, category 2): 16 days; group II B, category 1: mean, 17.0 days (21, 13, and 17 days); group II B, category 2: 14 and 15 days, respectively.

On the day before operation, less insulin than usual was given, in view of preoperative starvation. On the day of operation, no slow-acting insulin was given, but only regular insulin, divided into two small doses. Immediately after operation, every animal was given 20 ml of Aminosol-glucose solution (Vitrum) subcutaneously.

General Condition

The animals in category 1 generally stood the operation fairly well. They were killed after a postoperative observation period amounting to a mean 26.2 days in group I B (20, 28, 27, 28 and 28 days), and a mean 32.3 days in group II B (35, 35 and 27 days); thus a mean 28.5 days in the two groups collectively.

Complications of various kinds appeared in the three animals in category 2, so that the experiments could not be completed. Acidosis developed in R. 189; increased doses of insulin resulted in hypoglycaemic convulsions. The acidosis disappeared at the same time, but reappeared two days later. Death occurred 7 days after operation, and was presumed to be caused by diabetic acidosis. The bile flow had been consistently copious (414 ml in 7 days). R. 150 died of cholascos 2 days after operation. R. 175 was fairly strongly affected by operation but gradually improved. After a few days,

hypoglycaemic convulsions occurred, and the insulin dose was therefore reduced from 22 units preoperatively to 4 u/24 hrs. The condition nevertheless deteriorated successively, and death occurred 19 days after operation. Autopsy showed cholascos and liver necroses.

Bile Flow

In group I B (category 1), a continuous, copious bile flow was obtained until the end of the experiment in three animals (R. 14, 193 and 180). In the remaining two (R. 194 and 216), the bile flow ceased 12 days after operation, and the bag was empty thereafter. In association herewith, the animals became jaundiced and the test for bilirubin in the urine became strongly positive, as in the animals in group II. At autopsy, the lumen of the tube was found in both animals to be occluded close to the gallbladder by concretions.

Fig. 17 shows the 24-hour output of bile in this group in comparison to the conditions in group I A. The mean 24-hour output was 61.7 ml/24 hrs; thus, in this respect, the animals did not differ essentially from the non-diabetic animals.

The *electrolyte* and *glucose content* of the fistular bile was determined on altogether 16 occasions in 5 of the animals in group I B. The results are recorded in Table 6, from which it can be inferred that there was no difference between this group and group I A with respect to the content of potassium and chlorides, but that the sodium content was somewhat lower in the diabetic animals, the difference being probably significant. The bicarbonate content was considerably lower in the diabetic animals, the difference being highly significant. Replacement fluid of the same composition was given to the diabetic as to the non-diabetic animals (see p. 46). The mean glucose concentration in the bile of the diabetic animals amounted to 61.6 mg/100 ml. Since no determination of the biliary glucose was made in group I A, the implication of this value cannot be assessed, nor have I been able to find any statements in the literature regarding the normal concentration of glucose in the bile.

Body Weight

The change in BW in the animals of category 1 is shown in Fig. 36. During the preoperative diabetic period, three animals in group I B gained weight, one lost weight, and in one the weight remained unchanged. In group II B, one animal gained weight, and the other two lost weight.

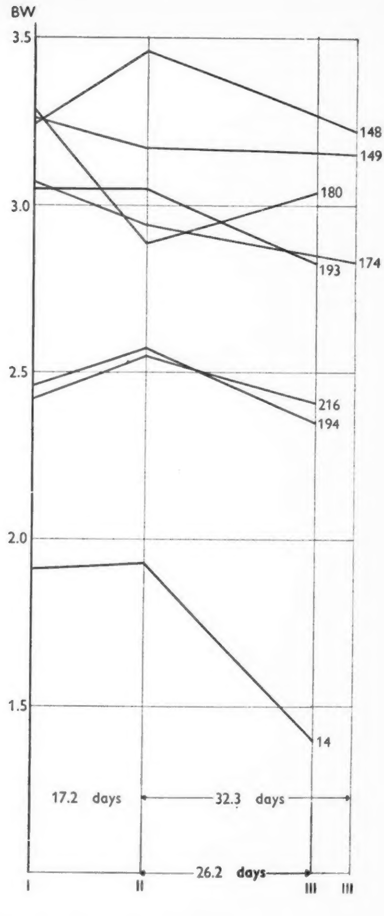

Fig. 36. Change in BW after alloxan administration and after bile flow intervention: groups I B and II B. I = alloxan administration. II = operation. III = death.

After operation, all the animals with the exception of R. 180 lost weight, the mean loss of weight in the two groups collectively amounting to 6.7 g/24 hrs, *i. e.*, a change in weight of about the same order of magnitude as in the corresponding non-diabetic animals.

DIABETIC CONDITION

The diabetic condition in the respective groups was evaluated by daily determinations of the glycosuria both pre- and postoperatively. As can be inferred from Tables 13 and 14, the mean preoperative glycosuria in

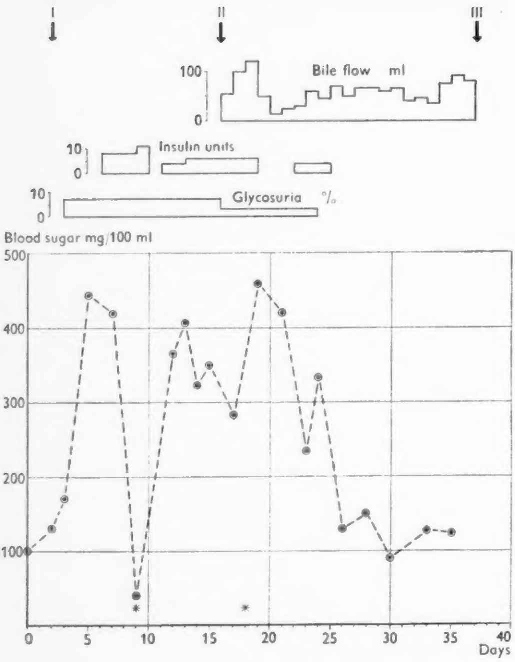

Fig. 37. Course of experiment in R. 14 (group I B).
I = alloxan administration.
II = operation. III = death.
** = hypoglycaemic convulsion.*

group I B was 12.6 g/24 hrs, and that in *group II B* 16.0 g/24 hrs. The 24-hour dose of insulin before operation was also about the same in both groups, *i. e.*, 14 to 15 units. As stated earlier, my intention was to give sufficient insulin to keep the glycosuria at about 10 g/24 hrs. It can be seen from Tables 13 and 14 that this was not invariably possible, the daily figure being above this level in several cases.

After operation, the glycosuria decreased to about half the earlier figure in both groups. Consequently, the insulin dose could also be reduced, and in three cases in group I B (R. 14, 193 and 194) it could be discontinued. One of these animals (R. 14) remained aglycosuric during the rest of the observation period, and in the two others the urinary glucose was about 1—3 g/24 hrs. The course in R. 14 is shown in Fig. 37. On two occasions this animal had hypoglycaemic convulsions. On the first occasion (before operation) a blood sample was taken during the convulsion, and the blood sugar was found to be 40 mg/100 ml; on the second occasion, no blood sample was taken during the convulsion. Hypoglycaemic convulsions were also observed in R. 193, 14 days after the biliary fistula operation.

TABLE 13

Group I B. Glycosuria and insulin treatment before and after biliary fistula operation
Period 1 = period of insulin treatment
Period 2 = period without insulin treatment

Animal no.	Glycosuria						Insulin treatment					
	Before operation		After operation Period 1		After operation Period 2		Before operation		After operation Period 1		After operation Period 2	
	No. of days	g/24 hrs	No. of days	g/24 hrs	No. of days	g/24 hrs	No. of days	Units/24 hrs	No. of days	Units/24 hrs	No. of days	Units/24 hrs
R. 14	14	+ + +	8	(+)	12	0	10	6.2	8	3.0	12	0
R. 193	11	14.95	14	3.80	14	2.93	10	19.2	14	4.0	14	0
R. 194	14	7.88	2	0.98	25	2.58	13	10.6	2	5.0	25	0
R. 216	33	10.85	28	7.21	—	—	32	8.1	28	4.0	—	—
R. 180	15	16.52	28	13.35	—	—	15	28.3	28	8.9	—	—
x̄	17	12.55	16	6.34	17	1.84	16	14.5	16	5.0	—	—

TABLE 14

Group II B. Glycosuria and insulin treatment before and after ligation of common bile duct

Animal no.	Glycosuria				Insulin treatment			
	Before operation		After operation		Before operation		After operation	
	No. of days	g/24 hrs	No. of days	g/24 hrs	No. of days	Units/24 hrs	No. of days	Units/24 hrs
R. 148	21	15.92	35	9.04	20	10.6	35	6.3
R. 149	13	10.01	35	8.72	12	8.2	35	3.9
R. 174	17	22.04	27	9.10	17	24.9	27	10.0
x̄	17	15.99	32	8.95	16	14.6	32	6.7

TABLE 15

Group IV G. Glycosuria and insulin treatment in alloxan-diabetic controls

Group	Animal no.	Glycosuria		Insulin treatment	
		No. of days	g/24 hrs	No. of days	Units/24 hrs
IV G a	R. 143	47	11.26	46	12.2
	R. 146	46	8.49	45	4.7
	R. 147	42	10.85	41	9.4
	R. 165	66	18.81	65	19.4
	x̄	50	12.35	49	11.4
IV G b	R. 243	30	21.38	29	20.1
	R. 245	30	16.63	29	18.3
	R. 246	29	24.67	28	18.4
	R. 247	30	11.42	29	7.7
	R. 248	30	16.00	29	16.3
	x̄	30	18.02	29	16.2
Both groups	x̄	39	15.50	38	14.1

The glycosuria and insulin doses in the alloxan-diabetic *control groups* IV G a and IV G b (category 1) are recorded in Table 15. The observation period was longer in the former group than in the latter. Thus, in group IV G a it was a mean 50.3 days, and in group IV G b a mean 29.8 days. The mean urinary excretion of glucose, as well as the size of the insulin dose required, were slightly lower in group IV G a than in group IV G b. The general condition of these animals was good during the whole observation period, and the mean gain in weight was 7.4 g/24 hrs in group IV G a,

the corresponding figure in group IV G b being 10.3 g/24 hrs. After the onset of diabetes, no essential change was observed in the degree of glycosuria or insulin requirement.

AUTOPSY

General Observations

The post mortem findings and cause of death in the animals in category 2 have already been described. The following observations were made in category 1. In the operated animals, a reduction in *adipose tissue* was noted, in similarity to the conditions in groups I A and II A. In four animals (R. 193, 194, 148 and 149) there was a total lack of adipose tissue, in one (R. 174) the lack was almost complete and in two (R. 14 and 216) the reduction was considerable. In R. 180 there was, on the contrary, no visible change in the quantity of adipose tissue.

Enlargement of the *liver* and cirrhosis, in some cases combined with ascites but without cholascos, was found in the animals of group II B, as well as in the two in group I B in which the bile flow had ceased, owing to occlusion of the tubing by concretions (R. 194 and 216). In R. 193, a well defined abscess cavity was present in one lobe of the liver; no signs of hepatic infection were observed in the other animals in group I B.

Nothing abnormal was found at autopsy of the controls in group IV G. Both the adipose tissue and the parenchymal organs had a normal appearance. Only in R. 246 were wide streaks of connective tissue present in the renal cortex. They may have been scars resulting from tubular damage caused by alloxan. Proteinuria had been present for a few days in this animal.

Pancreas Weight

The mean values of the total pancreas weight (PW) and of the pancreas weight per kg of body weight (PW/BW) are recorded in Table 17. The organ was not weighed in one case in group I B (R. 14). In the operated groups collectively, the common mean of the PW was 5.22 g; the corresponding figure in the control groups collectively was 4.15 g. The difference, 1.07 ± 0.44 g, is probably significant ($P \sim 0.02$, df $= 12$). The following means were obtained for the PW/BW: groups I B and II B collectively, 1.87 g; groups IV G a and IV G b collectively, 1.26 g. The difference, 0.61 ± 0.13 g, is highly significant ($P \sim 0.001$, df $= 12$). Thus, an increase in the size

of the pancreas seems to have taken place, as in the non-diabetic groups.

No difference was present in the PW or PW/BW in a comparison between groups I B and II B, nor between the PW in the two control groups. A significant difference was, on the contrary, found between the PW/BW in the two latter groups, the relative weight of the organ being greater in group IV G a. The cause of this condition is not definitely known but, as stressed earlier, the weight of the pancreas is not a particularly reliable measure of its size, owing to the variable quantity of intralobular fat.

QUANTITATIVE MICROMORPHOLOGIC ANALYSES

No quantitative micromorphologic analysis of the size of the total pancreas parenchyma was made in these groups.

The method for analysis of the islet tissue described in Chapter 3 was not used in R. 14. This animal belonged to a preliminary experimental series not included in the present material. The rest of the series comprised non-diabetic animals, in which the biliary fistula technique was tested. At autopsy, the pancreas of R. 14 was treated as follows.

The pancreas was divided into the D and the L part in the usual way. Part D was then divided into 5 pieces of equal size, and Part L into 6 pieces of equal size. A section was taken at random from each of these small pieces, and stained according to van Gieson. The average parenchymal area of these sections was considerably smaller than that obtained in the rest of the material.

About half the total number of islets present in the section were taken from each of the sections thus obtained for measurement of the islet size. The size of the whole sample, $S(N)$, was 388 islets, and $S(n_i)$ was 813. Calculations were then made of I_c, i, V_i, V_i/BW, m_i and n_i BW in the same way as in the rest of the material. The interval between the sections was counted as 1.2 mm, despite the fact that the actual interval was larger, although not known exactly. This approximation was considered justified, since it was evident that a considerably smaller portion of the organ had been examined in this case than in the others. Consequently, the value obtained for the islet volume could not, in any event, have been larger than if the analysis had been made in the same way as in the rest of the material. Despite this fact, the value obtained was of about the same order of magnitude as in the other animals in groups I B and II B, *i. e.*, considerably larger than in the alloxan-diabetic control groups. It could therefore be concluded that the islet volume had increased. The general appearance of the islets in this animal also indicated that this was, in fact, so; in most sections they were numerous and of normal size, without any of the features typical in the alloxan-diabetic controls.

Size of Islet Tissue

The results of the quantitative analyses of the islet tissue are assembled in Table 18 and Fig. 38. In a comparison with the figures obtained in the non-diabetic groups, it is evident that the order of magnitude is an entirely different one. It confirms the earlier known but not quantitatively esti-

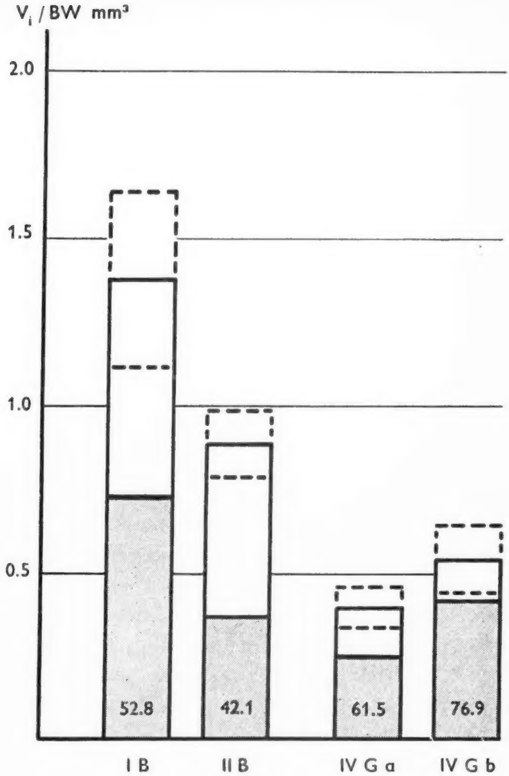

Fig. 38. Islet volume (V_i/BW): diabetic groups with functioning exocrine parenchyma. Mean values. ------ $x \pm e(x)$. *Shaded areas and figures in them denote mean alpha cell incidence in respective groups.*

mated fact that alloxan administration results in striking atrophy of the islets (DUFF; LUKENS). Thus, in these cases, the islets had decreased in both number and size.

Statistical analyses showed no difference between the two control groups with respect to n_i, n_i/BW, m_i, V_i or V_i/BW. This also applied to comparisons between groups I B and II B. It could be concluded from these findings that the difference in the mode of alloxan administration had not resulted in any difference as to the size of the islet tissue. Consequently, I considered it justified to make a statistical analysis of the differences between the operated groups collectively (groups I B and II B) and the control groups collectively (groups IV G a and IV G b). I then obtained df = 13. The difference between the length of the observation period in group IV G a and in group IV G b could scarcely have affected the results, since the

beta cell damage caused by alloxan leads to complete disappearance of the affected cells already within a few days (DE MOOR; DUFF; LUKENS).

These comparisons between the collected groups showed the difference to be highly significant with regard to n_i/BW and V_i/BW, but probably significant only in other respects (Table 16). In the operated animals, the increase in islet volume amounted to 91.3 per cent for V_i, and 150.0 per cent for V_i/BW.

Alpha and Beta Cell Count

The alpha cell incidence in the alloxan-diabetic controls was, as could be expected, high (*cf.* FERNER; GROBÉTY; THIEMER), even if fairly large individual variations were observed. The incidence was higher in group IV G b, the mean being 76.89 per cent, whereas the corresponding figure in group IV G a was 61.51 per cent; the difference is not significant.

In group I B, the alpha cell incidence could not be determined in two cases (R. 14 and 193). As mentioned earlier, no Gomori staining was done in the former animal; in the latter, staining was unsatisfactory, so that no distinct differentiation between alpha and beta cells was possible. Considerable variations in the alpha cell incidence were noted in the other three animals in group I B and the three in group II B, the range being 24 to 81 per cent. The mean value for these six animals was, however, 47.42 per cent; the difference between this value and that in the controls is probably significant. A tendency to normalization of the alpha to beta cell ratio was thus present.

HISTOLOGIC AND HISTOCHEMICAL EXAMINATION

Pancreas

The *general appearance* of the exocrine pancreas parenchyma was normal in every case, and no difference was present between the operated animals and the controls, apart from the general reduction in adipose tissue in the former.

Controls.—The islet tissue had, in addition to the quantitative changes already described, the appearance typical of alloxan diabetes, *i. e.*, fewer and smaller islets than normally, and a reversed alpha to beta cell ratio, with predominance of alpha cells. None of the numerous, very small islets entirely devoid of alpha cells that are present in the normal rabbit were observed in group IV G. The shape of the islets had also undergone

a change, and was more irregular than normally. Several small islets often lay close together and gave the impression of having originally belonged to a single large islet, in which connective tissue and exocrine parenchyma had invaded the site of the vanished beta cells. Figs. 39 and 40 show examples of such islets from animals in group IV G. In R. 247, the alloxan damage was not as conspicuous as in the other animals in this group, and the islet volume was somewhat larger. The insulin requirement had also been smaller in this animal than in most of the others.

Operated animals.—No such typical picture of alloxan damage was seen in these animals. Thus, in R. 194, the islet tissue had a practically normal appearance, with a fairly high incidence of relatively large islets, in which the alpha and beta cells were present in normal proportions (Fig. 41). Islet haemorrhage of the same type as in group II A was seen in some specimens from this animal. R. 180, on the other hand, exhibited a marked predominance of alpha cells, although the number of islets was considerably greater than in any other animal either in the operated or the control groups.

The appearance of the islet tissue was relatively uniform in the three animals of group II B. It contained a fairly large number of islets that bore a striking resemblance to the "Mantelinseln" in the embryonic pancreas described by FERNER & STOECKENIUS, *i. e.*, islets consisting of a core of beta cells and a continuous, peripheral "rind" of alpha cells, such as are not seen in the normal rabbit. In my cases, a break in the continuity of the peripheral alpha cell ring was, however, more often present than in the islets described by FERNER & STOECKENIUS. Examples of such islets are shown in Figs. 42, 43 and 44.

As may be seen in some of these figures, the alpha cells not only formed a peripheral rind about the islets. They were also present as a ring surrounding cross-cut intra-insular capillaries, directly beside the vessel wall, where they formed a kind of "barrier" between the vessel and the beta cells. In these cases, most of the beta cells were of the non-granular type. There is reason to point out that such cells denoted as beta cells may actually have been delta cells, since the Gomori stain does not permit differentiation between other kinds of cells than alpha and beta. According to BENCOSME, the majority of non-granular islet cells present in alloxan-diabetic rabbits are delta cells.

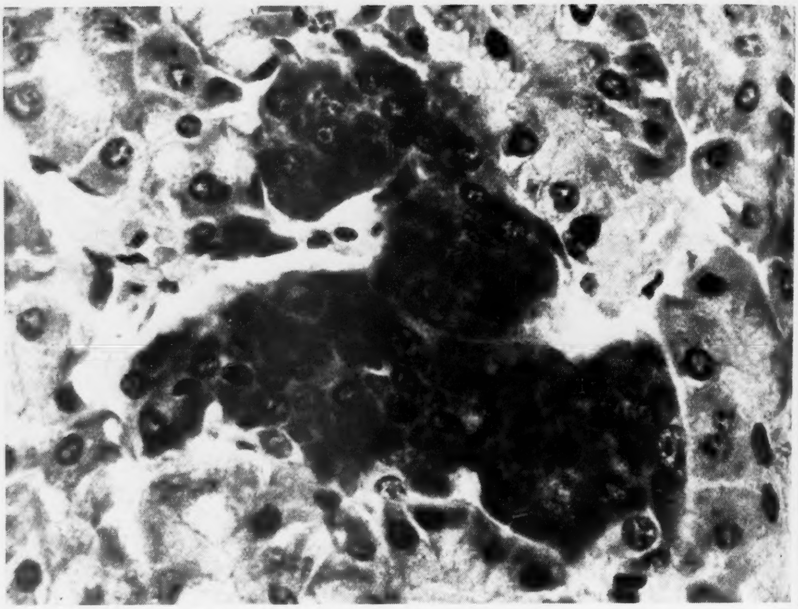

Fig. 39. Pancreas: alloxan-diabetic control (R. 147, group IV G a). Typical islet, consisting of alpha cells only. Gomori stain. Magnification 500 X.

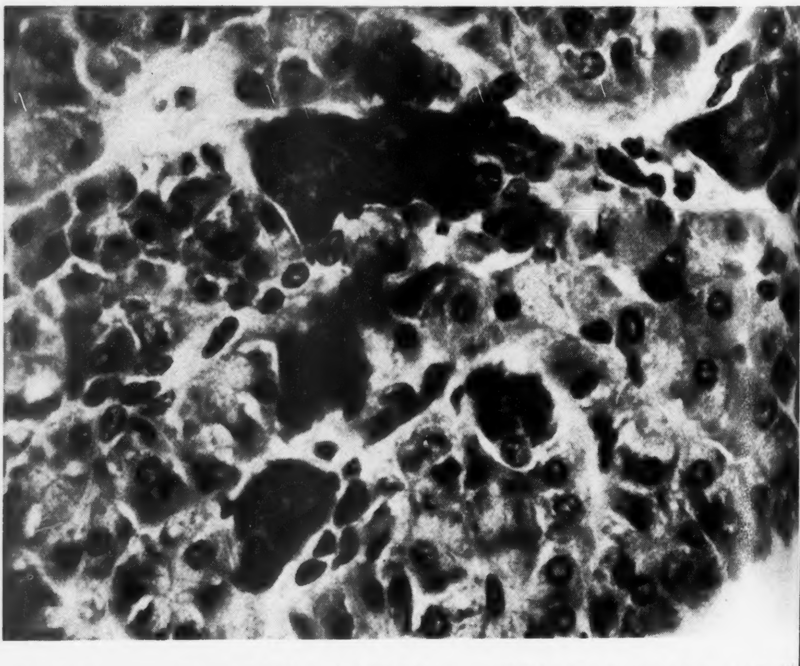

Fig. 40. Pancreas: alloxan-diabetic control (R. 246, group IV G b). Small, closely lying islets with irregular outlines, containing alpha cells only. Gomori stain. Magnification 500 X.

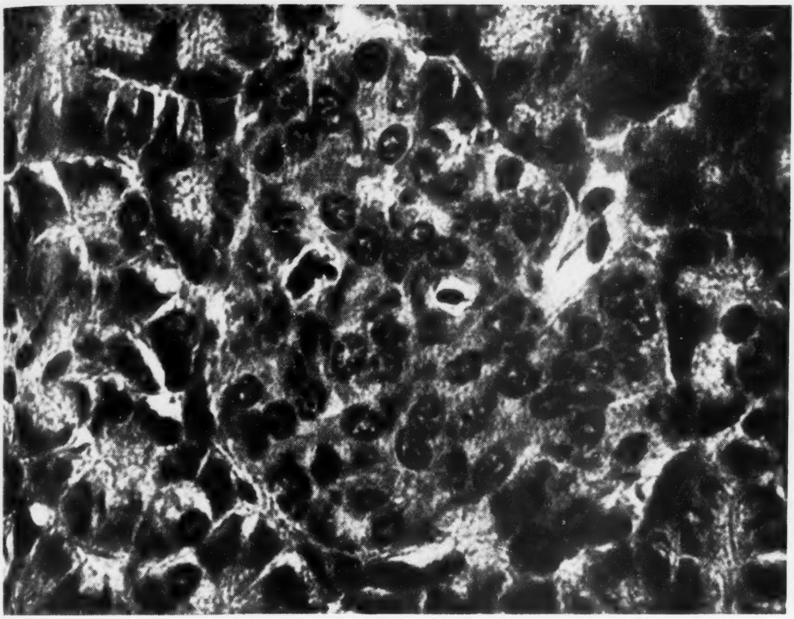

Fig. 41. Pancreas: alloxan-diabetic animal with biliary fistula (R. 194, group I B). Islet of normal appearance with predominance of beta cells and a few alpha cells in periphery. Gomori stain. Magnification 780 X.

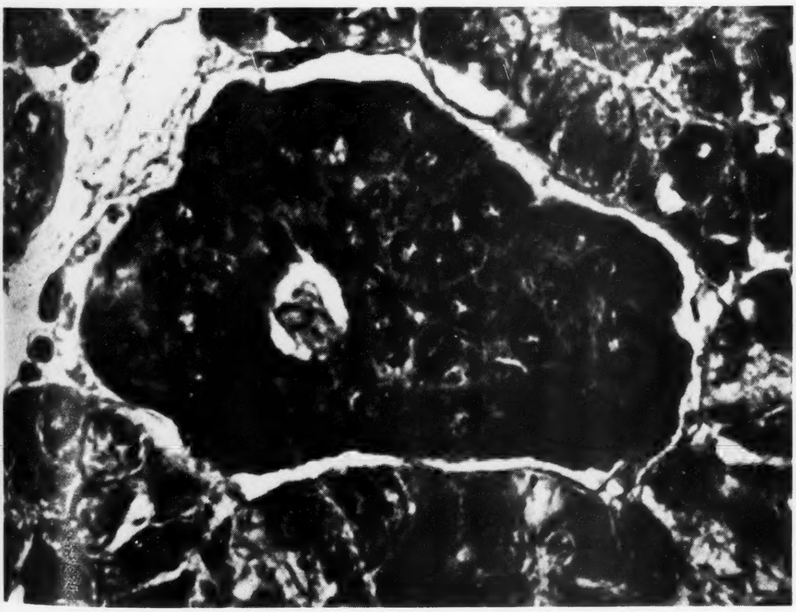

Fig. 42. Pancreas: alloxan-diabetic animal with biliary stasis (R. 148, group II B). Example of "Mantelinsel". Gomori stain. Magnification 980 X.

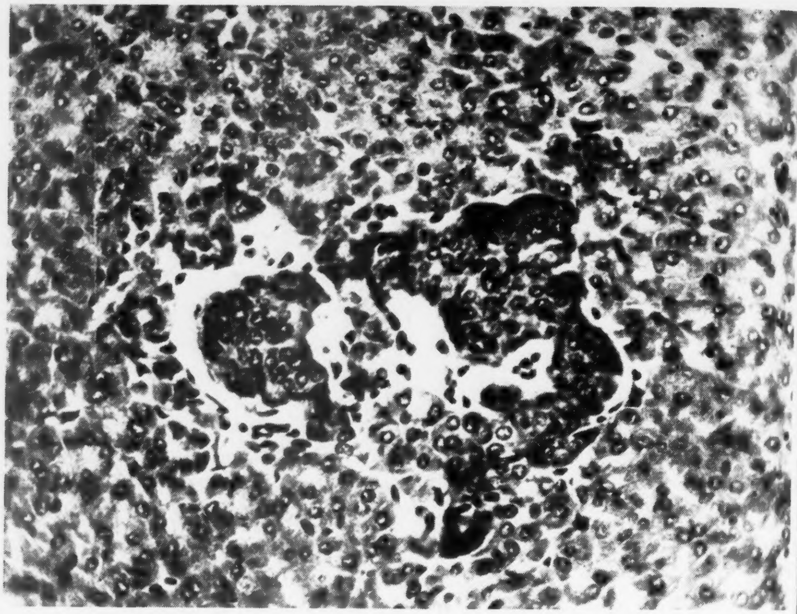

Fig. 43 Pancreas: alloxan-diabetic animal with biliary stasis (R. 148, group II B). Fairly large islet with high vascularity. Suggested formation of "Mantelinsel". Gomori stain. Magnification 300 X.

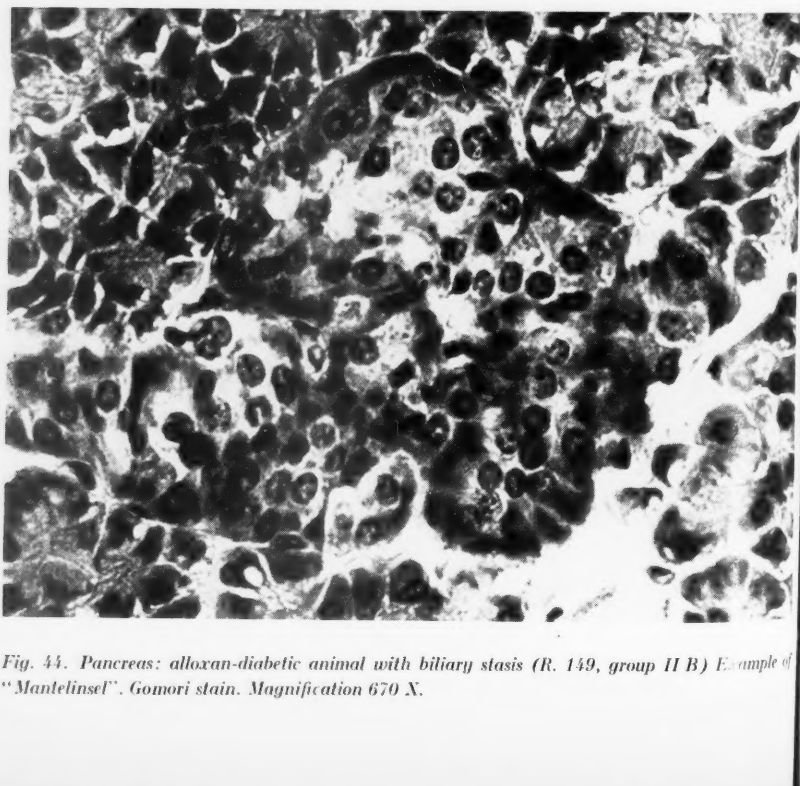

Fig. 44. Pancreas: alloxan-diabetic animal with biliary stasis (R. 149, group II B) Example of "Mantelinsel". Gomori stain. Magnification 670 X.

Other Organs

The *lipid content* of the *liver cells* was low in the animals of category 1 in groups I B and II B, with one exception (R. 194). The individual grading was: 0, 5, 1, 0; 0, 0, 0 (no histochemical analyses were made in R. 14). In the control groups, the grading was as follows: 4, 3, 5, 4; 5, 4, 5, 4, 6. The average was thus 0—1 in the operated animals and 4—5 in the controls, *i. e.*, a lipid content of the same order of magnitude as in the corresponding non-diabetic animals (*cf.* Table 12).

The *glycogen content* of the *liver cells* was graded as follows: Group I B: 4, 0, 6, 6; group II B: 6, 5, 4; control groups IV G: 4, 6, 7, 7; 7, 7, 7, 7, 7.

TABLE 16

Statistical comparison between operated and non-operated diabetic animals in groups I B, II B and IV G

Groups	I B + II B $\bar{x}$	IV G a + IV G b $\bar{x}$	Difference z	e(z)	t	P $\sim$
n_i	1,434	912	522	237.4	2.199	0.05*
n_i/BW	544	274	270	68.0	3.971	0.001***
m_i μ^2	1,815	1,425	390	179.4	2.174	0.05*
V_i mm³	3.06	1.60	1.46	0.53	2.755	0.02*
V_i/BW mm³	1.20	0.48	0.72	0.18	4.000	0.001***
Alpha cells %	47.42	69.98	22.56	8.03	2.809	0.02*

TABLE 17

Body size and pancreas size in diabetic groups with functioning exocrine parenchyma

		I B	II B	IV G a	IV G b
n		5 (4)	3	4	5
BW kg	$\bar{x}$	2.41	3.07	3.14	3.47
	$e(\bar{x})$	0.28	0.12	0.24	0.24
	s	0.63	0.21	0.49	0.54
BS dm²	$\bar{x}$	16.7	18.7	18.8	17.6
	$e(\bar{x})$	0.92	0.34	0.63	0.06
	s	2.05	0.59	1.27	1.35
PW g	$\bar{x}$	5.14	5.34	4.65	3.74
	$e(\bar{x})$	0.52	0.37	0.36	0.41
	s	1.04	0.64	0.72	0.92
PW/BW g	$\bar{x}$	1.95	1.75	1.48	1.07
	$e(\bar{x})$	0.21	0.16	0.02	0.07
	s	0.42	0.27	0.04	0.16

TABLE 18

Quantitative analysis of islet tissue in diabetic groups with functioning exocrine parenchyma

		I B	II B	IV G a	IV G b
n		5 (3)	3	4	5
l	$\bar{x}$	18.5	10.3	12.0	14.0
k	$\bar{x}$	8.8	7.0	6.5	6.6
N	$\bar{x}$	271	239	167	195
n_i	$\bar{x}$	1,495.6	1,330.3	784.8	1,013.4
	$e(\bar{x})$	367.9	136.8	67.1	107.0
	s	822.7	236.9	134.2	239.3
n_i/BW	$\bar{x}$	610.4	434.3	252.8	291.4
	$e(\bar{x})$	106.9	42.4	22.6	21.1
	s	239.1	73.5	45.2	47.2
m_i μ^2	$\bar{x}$	1,869.2	1,725.6	1,323.0	1,506.8
	$e(\bar{x})$	162.5	214.7	138.7	192.1
	s	363.3	371.8	277.3	429.6
V_i mm^3	$\bar{x}$	3.25	2.74	1.25	1.88
	$e(\bar{x})$	0.74	0.38	0.17	0.40
	s	1.66	0.66	0.34	0.89
V_i/BW mm^3	$\bar{x}$	1.38	0.89	0.40	0.54
	$e(\bar{x})$	0.26	0.10	0.06	0.10
	s	0.59	0.17	0.11	0.22
$\alpha + \beta$	$\bar{x}$	1,210	1,230	1,180	1,158
α %	$\bar{x}$	52.82	42.10	61.51	76.89
	$e(\bar{x})$	16.44	3.20	5.22	5.20
	s	28.47	5.54	10.44	11.63

The average was thus 4—5 in the operated animals, and 6—7 in the controls. The results differ from the findings in the non-diabetic groups in that the decreased liver glycogen content found in group II A was not recorded in diabetic animals with biliary stasis. Even if no definite explanation of this circumstance can be given, it seems probable that it is associated with the diabetic state of the last-mentioned animals, since the liver glycogen content of alloxan-diabetic animals has been stated to be raised (WARREN & LECOMPTE). It may also be related to any blood sugar

changes resulting from insulin therapy (*cf.* Blombäck *et al.*; Steincke; Swensson).—The glycogen content of the liver of animals in group IV G was the same as that in normally fed non-diabetic animals (groups IV A and IV D).

The *alkaline phosphatase activity* in the *liver cells* was graded as follows. Groups I B and II B: 6, 6, 7, 6; 1, 1, 5; group IV G: 6, 5, 6, 7; 6, 6, 6, 6, 4. The average was thus 4—5 in the operated groups and 5—6 in the control groups, *i. e.*, no definite difference between these groups, nor in relation to the non-diabetic animals.

The *phosphatase activity* of the *duodenal mucosa* and the *lipid content* of the *adrenal cortex* were the same in all the groups, and the same as in non-diabetic animals. As pointed out in Chapter 4, no glycogen infiltration in the renal tubules was observed in any of the animals. On the other hand there was, as in non-diabetic animals, a high glycogen content in the papillary ducts, close to their opening into the renal pelvis.

GROUP II C

The effect of alloxan in animals with biliary stasis was studied in two animals only (*group II C*). The salient features in these cases are given in the following animal records.

GROUP II C: CATEGORY 2

R. 231. Male. 1st obs. day 9/11 1954. BW 4.57 kg.—12/11 Operation: *Ligation of common bile duct.* BW 4.40 kg. Anaesthetic: ether + 300 mg kemithal.—14/11 Condition fairly good. Eats.—1/12 Glucose tolerance test. BW 4.11 kg. Glucose: 6.6 ml.—9/12 BW 4.06 kg. *Alloxan:* 100 mg/kg = 405 mg.—11/12 Massive glycosuria. No acetonuria or proteinuria. Condition good.—13/12 Blood sugar 366 mg/100 ml. BW 4.14 kg. Killed. *Autopsy:* generalized jaundice. Considerable but not total reduction in adipose tissue. Small quantity of clear yellow fluid in abdominal cavity. Bile ducts greatly dilated. Liver dark brown, firm and tough. Otherwise N.A.D. PW 7.11 g (D 4.48 g, L 2.63 g).—*Micr. exam.:* liver, biliary cirrhosis. No fat in liver cells.

R. 232. Male. 1st obs. day 9/11 1954. BW 3.84 kg.—12/11 Operation: *Ligation of common bile duct.* BW 3.50 kg. Anaesthetic: ether + 125 mg kemithal.—14/11 Condition good. Eats.—29/11 For past 24 hrs, somewhat more lethargic; appetite poorer. BW 3.25 kg. *Alloxan:* 100 mg/kg = 325 mg.—30/11 *Alloxan:* same dose as 29/11.—2/12 Glycosuria and acetonuria. Condition poor.—4/12 Glycosuria 2—3 %, corresponding to 2—4 g/24 hrs. Slight acetonuria. Proteinuria. Bilirubin test on urine positive. BW 2.84 kg. Killed. *Autopsy:* moderate but not total reduction in adipose tissue. Generalized jaundice. Rupture of gallbladder into omentum, which was dilated into thin-walled sac about 7 cm in diameter, containing bile-stained fluid. Liver enlarged, firm and tough. On surface of pancreas, fairly numerous small white spots (adipose tissue necrosis?). PW 5.98 g (D 3.12 g, L 2.86 g).—*Micr. exam.:* liver, biliary cirrhosis; fairly extensive large-dropped fatty degeneration of both cirrhotic connective tissue and liver cells.

These two animals were given an alloxan dose of 100 mg/kg of body weight on two successive days, starting on the 27th and 17th postoperative day, respectively. Glycosuria then appeared in both cases. Thus, ligation of the common bile duct did not protect these two rabbits against the diabetogenic action of alloxan.

DISCUSSION

The Diabetic Condition

It can be inferred from the aforegoing that a change took place in the operated animals with respect to glycosuria and insulin requirement, both being decreased. This change was distinct in all animals in category 1, but was most conspicuous in R. 14, in which complete normalization of the blood sugar and urine was recorded, despite no supply of exogenous insulin.

In all probability, this change was directly related to the *undernutrition* associated with the operations in question. The recognized clinical experience that undernutrition produces a decrease in hyperglycaemia and glycosuria in diabetic patients has been confirmed in alloxan-diabetic animals (*cf.* DE MOOR; MCCANDLESS *et al.*). The type of diet given to such animals can also affect the urinary output of glucose. Thus, BURN *et al.* found, in alloxan-diabetic rats, that a high-fat diet resulted in aglycosuria, whereas glycosuria reappeared on return to a normal diet. HOUSSAY & MARTINEZ also studied the dietary conditions in experimental diabetes, although their interest was mainly focused on the effect of alloxan and of subtotal pancreatectomy on animals given different types of diet for one month previously. They reported 100 per cent mortality in rats given alloxan after a high-fat diet, but only 40 per cent in animals fed a high-carbohydrate diet. Moreover, they found that subtotal pancreatectomy led to diabetes in 100 per cent of overfed animals, but in only 40 per cent of those underfed.

There is also reason to discuss whether the changes observed in the diabetic condition may have been an expression of *spontaneous changes* unrelated to the operations performed. It is known from earlier investigations that alloxan sometimes produces transient glycosuria only. However, in such cases, it has generally been administered in suboptimal doses (LUKENS). Moreover, the diabetic symptoms then usually start to regress shortly after alloxan has been given. Spontaneous late recovery from alloxan diabetes of more than a year's duration has, however, been described by LAZAROW in rats.

In my material, transient diabetes was present in one of the controls (R. 203). No signs of spontaneous improvement in the diabetic condition were observed in any of the other 18 controls. Nor did any of the operated animals exhibit any signs of spontaneous regression of glycosuria during the weeks preceding operation. Consequently, it seems possible to rule out the eventuality that the postoperative changes observed were due to spontaneous improvement unassociated with operation.

Even if the reduced caloric intake was the main cause of the observed changes in the diabetic condition, an *increased endogenous insulin production* may have been a contributory factor. Support is lent to this hypothesis by the results of the quantitative micromorphologic examination, *i. e.*, increased islet volume and a tendency to normalization of the alpha cell incidence.

Islet Morphology

Analyses of the islet tissue showed that its size was significantly greater in the operated animals than in the non-operated alloxan-diabetic controls, and that the alpha cell incidence was lower in the former than in the latter. The tendency was the same as that observed in non-diabetic rabbits that had undergone the same operations. Even if the causative mechanism may be the same in diabetic animals as in non-diabetic, the question of *regeneration* in alloxan diabetes is worth discussing.

It can be inferred from most earlier investigations that no definite regeneration of islet tissue has been observed in alloxan-diabetic animals. Thus, BARGMANN & CREUTZFELDT studied the pancreas of 28 dogs in which diabetes had been produced by varying doses of alloxan, up to 54 days after administration. They were unable to find any signs of new formation of beta cells, either from the alpha cells or from acinar tissue.

In view of the regeneration of islet tissue that can be observed in the remains of the gland after partial pancreatectomy in non-diabetic animals, CREUTZFELDT investigated the effect of this operation in alloxan-diabetic dogs. Signs of regeneration of islet tissue were found only to an inappreciable extent, and these regenerated islets contained chiefly alpha cells.

DUFF & TORESON have also stressed that, in their series of alloxan-diabetic rabbits with and without insulin therapy, there were no indications either of permanent improvement in the diabetic condition or of regeneration of beta cells.

In LAZAROW's aforementioned report of spontaneous recovery of alloxan-diabetic rats after long duration of the disease, it was pointed out that —despite this recovery—there were no definite signs of beta cell regeneration in the pancreas, although in a few cases a beta cell adenoma was

present. LAZAROW also referred to YOUNG's experience in two cats which recovered spontaneously from metahypophyseal diabetes. Despite this fact, the animals still exhibited signs of extensive hydropic beta cell degeneration. According to LAZAROW, such observations suggest that extra-pancreatic factors may be responsible for improvement of the diabetes.

The *guinea-pig* occupies a unique position in that it has not been possible to produce a permanent alloxan-diabetic condition in this animal (*cf.* DE MOOR). FERNER suggested as an explanation the special arrangement of the alpha cells close to the capillary walls in the islets of this animal, so that the alpha cells seem to form a protective barrier for the beta cells against alloxan. This hypothesis is contradicted by the investigations of JOHNSON. She was able to show that alloxan causes necrosis of the beta cells in the guinea-pig in the same way as in other animals, but that the absence of permanent diabetes is due to the fact that, within four days of alloxan administration, a complete regeneration of the islet tissue occurs by formation of many new, small islets, consisting entirely of beta cells.

According to the same author, this restoration of islet tissue is due to transformation of acinar cells into islet cells, and proliferation of the epithelium of the smallest ducts to form new small islets. Proliferation of pre-existing beta cells was considered to play a minor role in the replacement of the islet tissue.

OGILVIE described signs of islet regeneration in one alloxan-diabetic rabbit, treated with anterior pituitary extract. This isolated observation obviously requires confirmation. THIEMER failed to find any change in the alpha cell incidence in alloxan-diabetic rats treated with growth hormone. Nor did SUCHOWSKY observe any change in the islets of alloxan-diabetic rats after hypophysectomy.

To sum up, the following statement can be made. No definite signs have hitherto been found of regeneration of the islet tissue in alloxan-diabetic animals, with the exception of the guinea-pig, in which animal it has not been possible to produce permanent alloxan diabetes.

BARGMANN & CREUTZFELDT have suggested, as a possible cause of the lack of regeneration of islet tissue in alloxan diabetes, the extreme vacuolization of the epithelial cells in the intralobular pancreatic ducts, that was demonstrated in alloxan-diabetic dogs by GOLDNER & GOMORI. This naturally presupposes that regeneration must take place from this epithelium. Further evidence of such damage to the epithelium of the ducts was given by GROSSMAN & IVY. They believed that the intralobular duct cells might have an external secretory function, and that secretin might act on these cells. The secretin test was therefore made on four alloxan-

diabetic dogs; all of them were found to exhibit decreased responsiveness to secretin in relation to non-diabetic dogs. Labarre & Hanquinet also noted a change in the secretin effect in alloxan-diabetic dogs.

With the methods used in the present investigation, it was obviously impossible to elucidate the mechanisms of the islet regeneration observed. As stated earlier, it was not even possible to establish the nature of the non-granular islet cells observed in the operated animals, apart from the fact that they were not alpha cells. When discussing the origin of such newly-formed granular or non-granular islet cells, three alternatives seem to exist:

1. Formation from duct cells
2. Formation from alpha cells
3. Formation from remaining undamaged beta cells.

The first alternative is conceivable, in view of the role played by the duct epithelium in prenatal formation of islet cells. However—as already mentioned—the possibility of alloxan damage to this epithelium makes it uncertain whether these cells have retained the ability to form islet cells.

The second alternative is based on the hypothesis put forward by Ferner (1942), namely, that the alpha cells are inactive precursors to the beta cells. In view of later investigations on the nature and function of the alpha cells and their role in the production of glucagon, this hypothesis seems unlikely (*cf.* Korp & Lecompte). Nor was Bencosme able, in a recent study of the histogenesis and cytology of the islets in the rabbit embryo, to observe any signs of transition between alpha, beta and delta cells, which he concluded to be "cytogenetically independent".

The occurrence of "Mantelinseln" indicates that regenerative processes take place in the islets, since such islets have earlier been described mainly in the embryonic stage (Ferner & Stoeckenius). There are, however, no definite morphologic signs that formation of beta cells from alpha cells takes place in these islets.

The third alternative, regeneration of beta cells from the remaining, undamaged beta cells, thus seems to be the most probable explanation.

In none of the cases in my material did I observe any signs of new formation of alpha cells or of alpha cell syncytia, as described by Grobéty. Nor did I find, either in the operated animals or in the controls, indications of hydropic degeneration, which has been shown to be due to glycogen infiltration in the islet cells (Toreson). Duff & Toreson showed that this lesion is present only in animals not treated with insulin, and that it is independent of diabetic hyperglycaemia. It is therefore natural that this feature was lacking in my insulin-treated animals.

SUMMARY

Bile flow experiments were made in 11 alloxan-diabetic rabbits; in 6 cases a *biliary fistula* was created, and in 5 cases *ligation of the common bile duct* was performed. In two animals with a fistula, the bile flow ceased after 12 days, owing to the presence of concretions in the tubing. Because of the small number of surviving animals, and because, in non-diabetic animals, the effect on the pancreas of both biliary fistula and biliary stasis proved to be the same, the results of the two bile flow interventions are accounted for collectively in this chapter.

Operation was performed an average 17 days after the onset of diabetes. The mean post-operative observation period in 8 animals was 28.5 days.

The *course* and *post mortem findings* in these 8 cases are compared with the corresponding observations in 9 non-operated, alloxan-diabetic controls. Particular interest is then focused on the diabetic condition, and on the quantitative analysis of the islet tissue.

A *decrease in glycosuria* and in the *insulin requirement* was recorded in all the operated animals. In one of them, all diabetic symptoms disappeared. In two others, glycosuria was inappreciable, despite discontinuation of insulin. No essential change in glycosuria and insulin requirement was, on the contrary, observed in the controls. The change in the diabetic condition of the operated animals is discussed. It is suggested that the most probable cause is the reduced caloric intake postoperatively, possibly in combination with increased production of endogenous insulin.

Micromorphologic analysis of the *islet tissue* showed a significantly greater islet volume and a lower alpha cell incidence in the operated animals than in the controls. The increase in islet volume, expressed in mm per kg of body weight, amounted to 150 per cent in the operated animals in relation to the controls. Islet haemorrhage was observed in one animal. In three cases, numerous "Mantelinseln", as described by FERNER & STOECKENIUS, were present.

These observations are discussed against the background of earlier investigations on the possibilities of regeneration of islet tissue in alloxan diabetes. It is concluded that the most probable explanation of the increased incidence of beta cells, or non-granular islet cells, in the operated animals as compared to the controls is formation of new such cells from pre-existing beta cells, undamaged by alloxan.

Alloxan given to two animals with biliary stasis of 27 and 17 days' duration, respectively, produced diabetes in both cases.

CHAPTER 7

LIGATION OF PANCREATIC DUCT

A. NON-DIABETIC ANIMALS

Ligation of the pancreatic duct was performed in 14 non-diabetic animals: *group III A;* 10 (7 males and 3 females) were assigned to category 1, and 4 (all females) to category 2. This group thus consisted of the following animals:

Category 1: R. 177, 182, 183, 205, 206, 209, 210, 185, 186, 191

Category 2: R. 192, 208, 184, 139.

The reason for which the four animals were assigned to category 2 was in two cases premature death (R. 192, 208), in one case because the animal was found at operation to be pregnant (R. 184) and in one because the pancreatic duct could not be identified at operation (R. 139).

The controls were *group IV A*, consisting of 4 untreated, healthy animals (2 males and 2 females), and *group IV D*, consisting of 4 animals (2 males and 2 females) in which the left ureter was ligated. All the controls belonged to category 1, and comprised the following animals:

Group IV A: R. 178, 179, 241, 242

Group IV D: R. 152, 153, 154, 155.

The salient features of the course and the post mortem observations in these 22 experiments are given in the following animal records.

ANIMAL RECORDS

GROUP III A: CATEGORY 1

R. 177. Male. 1st obs. day 16/3 1954. BW 2.42 kg.—5/4 Glucose tolerance test. BW 2.09 kg. Glucose: 3.4 ml.—7/4 Operation: *Ligation of pancreatic duct.* BW 2.16 kg. Anaesthetic: ether.—9/4 Condition good. Eats.—9/6 Glucose tolerance test. BW 2.50 kg. Glucose: 4.0 ml.—10/6 Condition good. No glycosuria. BW 2.62 kg. Killed. *Autopsy:* complete atrophy of pancreas; ducts greatly dilated. Otherwise N.A.D. PW 2.10 g (D 1.43 g, L 0.67 g).

R. 182. Male. 1st obs. day 26/3 1954. BW 2.17 kg.—12/4 Operation: *Ligation of pancreatic duct.* BW 2.28 kg. Anaesthetic: ether.—14/4 Condition good. Eats.—9/6 Glucose tolerance test.

BW 2.58 kg. Glucose: 4.1 ml.—10/6 Condition good. No glycosuria. BW 2.70 kg. Killed. *Autopsy:* complete atrophy of pancreas; ducts greatly dilated. Otherwise N.A.D. PW 2.38 g (D 1.34 g, L 1.04 g).

R. 183. Male. 1st obs. day 26/3 1954. BW 2.46 kg.—12/4 Operation: *Ligation of pancreatic duct.* BW 2.48 kg. Anaesthetic: ether.—14/4 Condition good. Eats.—18/6 Glucose tolerance test. BW 2.92 kg. Glucose: 4.7 ml.—20/6 Condition good. No glycosuria. BW 2.99 kg. Killed. *Autopsy:* complete atrophy of pancreas; ducts greatly dilated. Otherwise N.A.D. PW 2.70 g (D 1.68 g, L 1.02 g).

R. 205. Male. 1st obs. day 30/7 1954. BW 2.08 kg.—2/8 Operation: *Ligation of pancreatic duct.* BW 2.25 kg. Anaesthetic: ether.—4/8 Condition good. Eats.—11/10 Glucose tolerance test. BW 2.63 kg. Glucose: 4.2 ml.—25/10 Condition good. No glycosuria. BW 2.82 kg. Killed. *Autopsy:* complete atrophy of pancreas; ducts greatly dilated. Otherwise N.A.D. PW 5.49 g (D 3.93 g, L 1.56 g).

R. 206. Male. 1st obs. day 30/7 1954. BW 2.16 kg.—2/8 Operation: *Ligation of pancreatic duct.* BW 2.25 kg. Anaesthetic: ether.—4/8 Condition good. Eats.—11/10 Glucose tolerance test. BW 2.51 kg. Glucose: 4.0 ml.—25/10 Condition good. No glycosuria. BW 2.61 kg. Killed. *Autopsy:* pancreas mainly atrophied, with considerable dilatation of ducts. In duodenal part close to pylorus, pancreas parenchyma of normal appearance in an area of about 2 cm². On surface of kidneys, close-lying spots 0.5 to 1 mm in diameter, of firmer consistency than surroundings; similar small areas on cut surface of cortex. Otherwise N.A.D. PW 6.59 g (D 4.76 g, L 1.83 g).—*Micr. exam.:* in cortical areas of kidneys, corresponding to macroscopic changes, strands and foci of connective tissue with plentiful lymphocytes; they partly split up the otherwise normal parenchyma. The macroscopic observation that a small proportion of the D part of the pancreas had escaped atrophy was confirmed. The islets in this part were not included in calculation of the islet volume.

R. 209. Male. 1st obs. day 30/7 1954. BW 1.77 kg.—4/8 Operation: *Ligation of pancreatic duct.* BW 1.94 kg. Anaesthetic: ether.—6/8 Condition good. Eats.—13/10 Glucose tolerance test. BW 2.33 kg. Glucose: 3.7 ml.—27/10 Condition good. No glycosuria. BW 2.44 kg. Killed. *Autopsy:* complete atrophy of pancreas; ducts greatly dilated. Otherwise N.A.D. PW 4.88 g (D 3.15 g, L 1.73 g).

R. 210. Male. 1st obs. day 30/7 1954. BW 1.97 kg.—4/8 Operation: *Ligation of pancreatic duct.* BW 2.08 kg. Anaesthetic: ether.—6/8 Condition good. Eats.—13/10 Glucose tolerance test. BW 2.58 kg. Glucose: 4.1 ml.—27/10 Condition good. No glycosuria. BW 2.67 kg. Killed. *Autopsy:* complete atrophy of pancreas; ducts greatly dilated. Otherwise N.A.D. PW 5.39 g (D 3.28 g, L 2.11 g).

R. 185. Female. 1st obs. day 4/5 1954. BW 2.13 kg.—7/5 Operation: *Ligation of pancreatic duct.* BW 1.98 kg. Anaesthetic: ether.—9/5 Condition good. Eats.—29/6 Glucose tolerance test. BW 2.38 kg. Glucose: 3.8 ml.—2/7 Condition good. No glycosuria. BW 2.51 kg. Killed. *Autopsy:* complete atrophy of pancreas; ducts greatly dilated. Otherwise N.A.D. PW 4.38 g (D 2.81 g, L 1.57 g).

R. 186. Female. 1st obs. day 4/5 1954. BW 3.42 kg.—5/5 No glycosuria.—11/5 Operation: *Ligation of pancreatic duct.* BW 3.30 kg. Anaesthetic: ether.—13/5 Condition good. Eats.—29/6 Glucose tolerance test. BW 3.30 kg. Glucose: 5.3 ml.—1/7 Massive glycosuria: 4.5 %, 11.0 g.—2/7 Glycosuria: 5.1 %, 8.4 g.—3/7 Glycosuria: 5.7 %, 13.7 g. Blood sugar 344 mg/100 ml. Condition good. No acetonuria.—23/7 Glycosuria: 1.2 %, 2.5 g. Blood sugar 221 mg/100 ml.—24/7 Glycosuria 3.3 %, 5.8 g.—26/7 Glycosuria 0.3 %, 1.8 g.—27/7 Condition consistently unaffected, appetite normal. Scarcely measurable glycosuria. No acetonuria. BW 3.51 kg. Killed.

Autopsy: complete atrophy of pancreas; ducts greatly dilated. Otherwise N.A.D. PW 4.63 g (D 3.13 g, L 1.50 g).

R. 191. Female. 1st obs. day 18/5 1954. BW 2.28 kg.—11/6 Operation: *Ligation of pancreatic duct.* BW 2.74 kg. Anaesthetic: ether.—13/6 Condition good. Eats.—27/7 Glucose tolerance test. BW 3.35 kg. Glucose: 5.4 ml (blood sugar determinations unsuccessful).—16/8 Condition good. No glycosuria. BW 3.94 kg. Killed. *Autopsy:* atrophy of pancreas, apparently complete, but a small portion in pylorus region did not have entirely atrophic appearance. Ducts greatly dilated. Otherwise N.A.D. PW 6.96 g (D 4.11 g, L 2.85 g).—*Micr. exam.:* no functioning exocrine parenchyma in pancreas.

GROUP III A: CATEGORY 2

R. 192. Female. 1st obs. day 18/5 1954. BW 2.28 kg.—11/6 Operation: *Ligation of pancreatic duct.* BW 2.84 kg. Anaesthetic: ether.—12/6 Paralysis of hind quarters, otherwise fairly lively.—16/6 Condition worse; paralysis of bladder and rectum.—17/6 Died. *Autopsy:* BW 2.52 kg. Plentiful turbid fluid in abdominal cavity; fibrin deposits on intestines. Pancreatic ducts dilated; incipient atrophy of parenchyma. Paralysis seemed to be due to fracture of a vertebra with spinal cord injury, probably in course of operation.—No *micr. exam.* made.

R. 208. Female. 1st obs. day 30/7 1954. BW 1.77 kg.—3/8 Operation: *Ligation of pancreatic duct.* BW 1.71 kg. Anaesthetic: ether.—5/8 Condition good. Eats.—17/8 Despite good condition and appetite, considerable postoperative loss of weight.—18/8 Found dead in morning. *Autopsy:* BW 1.14 kg. Cachexia. Atrophy of pancreas; ducts dilated. Otherwise N.A.D. Cause of death could not be definitely established; probably some form of nutritional disturbance resulting from operation.—No *micr. exam.* made.

R. 184. Female. 1st obs. day 4/5 1954. BW 2.64 kg.—7/5 Operation: *Ligation of pancreatic duct.* BW 2.58 kg. Anaesthetic: ether. Found at operation to be pregnant. Although experiment of no interest for this reason, operation completed.—22/5 Parturition: 6 in litter; 5 living, 1 dead.—24/5 Whole litter dead.—30/6 BW 2.68 kg. Killed. *Autopsy:* complete atrophy of pancreas; ducts dilated. Otherwise N.A.D. PW 3.82 g (D 2.97 g, L 0.85 g).

R. 139. Female. 1st obs. day 2/10 1953. BW 2.35 kg.—6/10 Glucose tolerance test. BW 2.50 kg. Glucose: 4.0 ml.—24/11 Operation: *Ligation of pancreatic duct.* BW 3.16 kg. Anaesthetic: 285 mg kemithal. Pancreatic duct could not be definitely identified; tissue of duct-like nature divided.—26/11 Condition good. Eats.—8/1 Glucose tolerance test. BW 3.50 kg. Glucose: 5.6 ml.—12/1 BW 3.63 kg. Killed. *Autopsy:* pancreas of normal appearance; no signs of atrophy. Otherwise N.A.D. PW 5.12 g (D 2.41 g, L 2.71 g).

GROUP IV A: CATEGORY 1

R. 178. Female. 1st obs. day 16/3 1954. BW 2.48 kg.—27/4 Glucose tolerance test. BW 3.10 kg. Glucose: 5.0 ml.—13/5 BW 3.20 kg. Condition and appetite normal throughout observation period. Killed. *Autopsy:* N.A.D. PW 5.29 g (D 3.26 g, L 2.03 g).

R. 179. Female. 1st obs. day 16/3 1954. BW 2.46 kg.—27/4 Glucose tolerance test. BW 3.00 kg. Glucose: 4.8 ml.—13/5 BW 3.02 kg. Condition and appetite normal throughout observation period. Killed. *Autopsy:* N.A.D. PW 3.84 g (D 2.50 g, L 1.34 g).

R. 241. Male. 1st obs. day 25/1 1955. BW 2.46 kg.—17/2 BW 2.67 kg. Condition and appetite normal throughout observation period. Killed. *Autopsy:* N.A.D. PW 3.79 g (D 2.25 g, L 1.54 g).

R. 242. Male. 1st obs. day 25/1 1955. BW 2.97 kg.—17/2 BW 3.28 kg. Condition and appetite normal throughout observation period. Killed. *Autopsy:* N.A.D. PW 4.30 g (D 2.24 g, L 2.06 g).

GROUP IV D: CATEGORY 1

R. 152. Male. 1st obs. day 5/1 1954. BW 2.54 kg.—18/1 Operation: *Ligation of left ureter.* BW 2.58 kg. Anaesthetic: ether.—24/2 Condition and appetite consistently good. BW 2.85 kg. Killed. *Autopsy:* quantity and appearance of adipose tissue normal throughout. Left kidney hydronephrotic; contained dark brown, turbid fluid. Otherwise N.A.D. PW 3.89 g (D 2.57 g, L 1.32 g).—*Micr. exam.:* tubules of left kidney greatly dilated, with low epithelium; considerable increase in connective tissue. Right kidney: N.A.D. Phosphatase activity demonstrable in a few epithelial cells of left kidney; in right kidney to normal extent.

R. 153. Male. 1st obs. day 5/1 1954. BW 2.39 kg.—18/1 Operation: *Ligation of left ureter.* BW 2.45 kg. Anaesthetic: ether.—10/2 Glucose tolerance test. BW 2.46 kg. Glucose: 3.9 ml.—24/2 Condition and appetite consistently good. BW 2.61 kg. Killed. *Autopsy:* quantity and appearance of adipose tissue normal throughout. Left kidney hydronephrotic; contained dark brown, turbid fluid. Otherwise N.A.D. PW 3.96 g (D 2.77 g, L 1.19 g).—*Micr. exam.:* tubules of left kidney greatly dilated, with low cubical epithelium; considerable increase in connective tissue. Right kidney: N.A.D. Moderate phosphatase activity in epithelial remains of left kidney; in right kidney to normal extent.

R. 154. Female. 1st obs. day 5/1 1954. BW 2.83 kg.—8/1 Glucose tolerance test. BW 2.72 kg. Glucose: 4.4 ml.—11/1 Operation: *Ligation of left ureter.* BW 2.73 kg. Anaesthetic: 250 mg kemithal.—10/2 Glucose tolerance test. BW 3.12 kg. Glucose: 5.0 ml.—22/2 Condition and appetite consistently good. BW 3.53 kg. Killed. *Autopsy:* quantity and appearance of adipose tissue normal throughout. Left kidney hydronephrotic; contained brown, turbid fluid. Otherwise N.A D. PW 6.77 g (D 5.22 g, L 1.55 g).—*Micr. exam.:* tubules of left kidney greatly dilated, with low cubical epithelium; considerable increase in connective tissue. Right kidney: N.A.D. Slight phosphatase activity in a few epithelial cells of left kidney; in right kidney to normal extent.

R. 155. Female. 1st obs. day 5/1 1954. BW 2.98 kg.—13/1 Operation: *Ligation of left ureter.* BW 2.90 kg. Anaesthetic: ether.—22/2 Condition and appetite consistently good. BW 3.26 kg. Killed. *Autopsy:* quantity and appearance of adipose tissue normal throughout. Left kidney hydronephrotic; contained fairly plentiful yellowish-white, turbid fluid. Otherwise N.A.D. PW 5.57 g (D 3.66 g, L 1.91 g).—*Micr. exam.:* great induration of connective tissue of left kidney; no visible remains of tubules. Right kidney: N.A.D. No phosphatase activity in left kidney; in right kidney to normal extent.

POSTOPERATIVE COURSE

Observation Period

In group III A, the mean observation period for the animals in category 1 was 72.7 days (64, 59, 69, 84, 84, 84, 84, 56, 77 and 66 days). In category 2, the corresponding figures were 31.0 days (6, 15, 49 and 54 days). The animals in group IV A were under observation for a mean 40.0 days before they were killed, the individual figures being 57, 57, 23 and 23 days. In group IV D, the mean postoperative observation period amounted to 39.0 days (37, 37, 42 and 40 days).

General Condition

The animals in group III A, category 1, stood the operation well, and started to eat already a few hours after it. The behaviour of these animals did not subsequently differ in any respect from that of untreated healthy animals.

As far as the four animals in category 2 are concerned, paralysis of the hind quarters (operative trauma), including bladder and rectal paralysis, appeared in R. 192. The animal died 6 days after operation, with signs of peritonitis. R. 208 died in a state of cachexia 15 days after operation. This was the only one of all the 33 animals in the material, in which ligation of the pancreatic duct was successfully performed, to exhibit a postoperative nutritional disturbance. It cannot be determined whether this was in some way to be ascribed to the cessation of external pancreatic function.

R. 184 was excluded from the quantitative micromorphologic analysis, in view of the possible influence of pregnancy on the islet tissue. R. 139 was killed 54 days after operation and was found to have a completely intact pancreas. In this case, it was doubtful whether the pancreatic duct had been identified at operation (*cf.* Animal Records).

In group IV D, the animals stood the operation well, and exhibited no signs of illness during the subsequent course; their appetite was normal and they gained in weight. The general condition of the animals in group IV A was normal throughout the observation period.

Body Weight

All the animals in category 1 gained weight during the observation period. Fig. 45 shows the weight conditions in group III A. The gain in weight was about the same in all the animals, except in R. 191, in which it was considerably greater than in the others. The mean gain in this group was 7.6 g/24 hrs.

In group IV D, the mean postoperative gain in weight was 398 g, *i. e.*, 9.9 g/24 hrs; in group IV A, the corresponding figures were 450 g and 11.3 g/24 hrs. By way of comparison, it may be recalled that 18 animals in other groups, under observation for a mean 39.2 days before starting the relevant experiments, gained a mean 506 g, or 11.2 g/24 hrs, during this period (*cf.* Chapter 4, p. 60). This gain in weight is the same as in normal rabbits of corresponding age and weight, given a normal diet.

A comparison between the mean values for the body weight in group III A and in the two control groups collectively showed no difference; this also applied to a comparison between groups IV A and IV D.

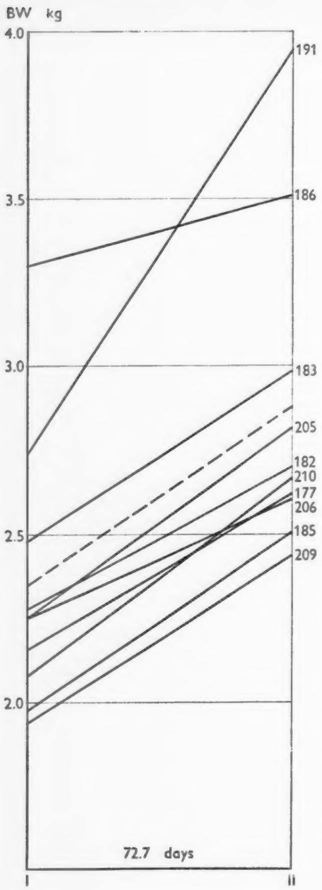

Fig. 45. Change in BW during postoperative observation period: group III A (ligation of pancreatic duct). — — — mean value. I = operation. II = death.

Diastasuria

Diastasuria is known to appear in connexion with ligation of the pancreatic duct. Its extent is shown in Table 19. (It is true that this table was constructed on the basis of results obtained in animals in group III C b, but at this stage these animals were fully comparable with those in group III A.) It is evident from Table 19 that although the diastasuria was high during the first 24 to 48 hours after operation, it decreased rapidly thereafter and had completely disappeared 4 to 6 days postoperatively. This seems to indicate that the enzyme production of the acinar parenchyma ceases soon after obstruction of the duct.

TABLE 19

Diastasuria (Wohlgemuth units) before and after ligation of pancreatic duct (group III C b)

Animal no.	Before operation (days)		After operation (days)										
	2	1	1	2	3	4	5	6	7	8	9	10	11
R. 235	16	8	512	256	256	256	128	128	64	32	8	—	8
R. 236	8	8	32	256	256	128	32	4	4	8	4	—	8
R. 237	4	64	1,024	512	64	16	16	4	4	—	—	8	—
R. 238	16	16	512	256	32	32	32	8	32	—	—	16	—
R. 239	2	8	256	256	32	32	16	8	4	—	—	—	4
R. 240	16	8	128	256	128	64	16	4	16	—	—	—	32
$\bar{x}$	8	16	256	256	128	64	32	8	16	16	8	16	8

GLUCOSE TOLERANCE TESTS

In group III A, glucose tolerance tests were made postoperatively in all the animals of category 1 except R. 191. The test was made a mean 65.1 days after operation. The results are recorded in Table I: 8. A curve pathologic in every respect was obtained in one case (R. 186). The fasting value was 208 mg/100 ml, the maximum value was noted 10 minutes after injection and was 302 mg/100 ml. The level then fell slowly to 226 mg/100 ml at 90 min. The tolerance area was thus considerable, *i. e.*, 16,385 mg-min. This was obviously a "diabetic" type of response to the glucose injection, and glycosuria was present at the time. The glycosuria, which was maximally about 6 per cent was not, however, associated with acetonuria, nor was the general condition of the animal affected. The glycosuria decreased successively, and was less than 0.5 per cent when the animal was killed about 4 weeks after the glucose tolerance test had been made. Since this animal differed so greatly from the others in this group with respect to glucose tolerance, it was not included in calculation of the mean tolerance area for the group. This mean amounted to 4,957 mg-min, and thus did not differ significantly from the mean value of 4,784 mg-min in the "normal group" (*cf.* Table 26).

In group IV D, a postoperative glucose tolerance test was made in R. 153 and R. 154 only. Any intrinsic effect of operation on the glucose tolerance could, however, also be evaluated from the postoperative tolerance test made in R. 139 (group III A, category 2) and in R. 163 (group II A, cate-

gory 2). As stated earlier, the planned intervention had been unsuccessful in these cases, in the former owing to failure to identify the pancreatic duct, and in the latter owing to recanalization of the common duct. The glucose tolerance in group IV D was thus evaluated on the basis of four tests (Table I: 10). The mean value of the tolerance area thus obtained, 5,330 mg-min, did not differ significantly from that in the "normal group" (*cf.* Table 26).

AUTOPSY

At autopsy of the animals in group III A (category 1), operation was found in every case to have produced the intended effect, *i. e.*, complete atrophy of the pancreas parenchyma. Thus, at the former site of the organ, only the large ducts were present; they were dilated to a varying degree and generally contained transparent, colourless fluid with a number of small white concretions. The ducts were imbedded in more or less plentiful fat. R. 206 was an exception; in this case, an extremely small remains of the gland was found in the vicinity of the pylorus, and had no communication with the otherwise atrophic pancreas. The secretory product of this remains obviously emptied into the duodenum through one or several small, accessory ducts. The existence of such ducts, and complete isolation of the part of the gland supplied by these ducts from the main part of the organ, were already demonstrated by CLAUDE BERNARD. R. 206 was, however, the only animal in this material in which the existence of such accessory ducts was observed.

Despite this atrophy of the pancreas, I dissected out the fat-infiltrated remains with the same technique as that used in animals with functioning exocrine parenchyma. Although, under such conditions, the weight of the organ is of no great interest, it is given in the animal records and tables in order to provide an approximate idea of the size of the portion of tissue removed. As may be inferred from the tables, this portion weighed about the same as in the groups with functioning exocrine parenchyma, the mean values in group III A being 4.55 g for PW, and 1.59 g for PW/BW.

In group IV D, the effect of operation could be confirmed at autopsy. In every case the left kidney was hydronephrotic, and no remains of normal structures were visible microscopically.

In group IV A, the mean value of PW was 4.31 g and that of PW/BW was 1.41 g; the corresponding figures in group IV D were 5.05 g and 1.63 g. The difference between the groups is not significant.

QUANTITATIVE MICROMORPHOLOGIC ANALYSES

Size of Total Pancreas Parenchyma

No micromorphologic analyses were made of the inappreciable remains of the pancreas parenchyma observed in R. 206. In the other animals in group III A, there was obviously no parenchyma left for measurement. The size of the parenchyma was, however, measured in the animals in the control groups; the results are shown in Table 10 and Fig. 32 (Chapter 5). In group IV A, the mean value of V_p was 1,311.3 mm³ and that of V_p/BW was 432.5 mm³. The corresponding means in group IV D were 1,148.7 mm³ and 377.5 mm³. There is no significant difference between the groups in these respects.

Size of Islet Tissue

The results of the quantitative analyses of the islet tissue in group III A are recorded in Table 25, and those in control groups IV A and IV D in Table 11. The results are also shown in Figs. 52 and 34. The following findings were made.

A difference was present between the two control groups in that the islet tissue was somewhat larger in the untreated group IV A than in group IV D, in which the left ureter was ligated. The difference is probably significant with respect to n_i, n_i/BW and V_i, and significant with respect to V_i/BW. No difference between the groups was, on the contrary, present in the case of m_i. In view of this difference between the control groups, I did not consider it justified to compare the operated group III A with the control groups collectively. Comparisons were made instead between the operated group and each of the control groups separately (Table 20).

These comparisons showed that no difference existed between groups III A and IV A with respect to the number of islets (n_i and n_i/BW), but that group IV A had significantly higher figures for islet size (m_i) and calculated islet volume (V_i and V_i/BW). In relation to group IV D, group III A had a greater number of islets (n_i and n_i/BW), the difference being probably significant, but islet size and islet volume were greater in group IV D, the differences being significant.

In other words, the number of islets remained unchanged or increases, whereas the islet size diminished considerably, resulting in a decrease in the calculated islet volume as well. Expressed as the percentages, the decrease in V_i/BW in group III A in relation to group IV A amounted to 54.7 per cent, and in relation to group IV D to 39.7 per cent. The lowest

TABLE 20

Statistical comparison between mean size of islet tissue in groups III A, IV A and IV D

Groups	df	n_i	n_i/BW	m_i	V_i	V_i/BW
III A—IV A	12	0	0	IV A ***	IV A ***	IV A ***
III A—IV D	12	III A *	III A *	IV D ***	IV D ***	IV D *
IV A—IV D	6	IV A *	IV A *	0	IV A *	IV A **

0 = no difference
* = probably significant difference
** = significant difference
*** = highly significant difference

When a difference is present, the group in which the mean is higher is given in the relevant column

value for islet volume was found in R. 186, V_i being 1.58 mm³ and V_i/BW 0.45 mm³. This fact, in addition to the alpha cell count (31.09 per cent), which was the highest in the whole group, seems to explain the glycosuria and diabetic type of glucose tolerance exhibited by this animal.

Alpha and Beta Cell Count

In group III A, the alpha cell incidence was on the whole normal, the mean being 19.82 per cent. If the exceptional value recorded in R. 186 is ruled out, the scattering in the group was moderate, with a range of 13 to 25 per cent. The mean value for the alpha cell incidence in group IV A was 20.10 per cent, and that in group IV D was 18.85 per cent. Thus, the operated group did not differ from the control groups in this respect.

HISTOLOGIC AND HISTOCHEMICAL EXAMINATION

Pancreas

The histologic appearance of the pancreatic tissue in group III A was typical, and entirely in agreement with that described by earlier workers. Thus, the glandular parenchyma was replaced by adipose and connective tissue, in which were seen dilated ducts with proliferation of their epithelium, and the islets of Langerhans pressed between them. Large islets were

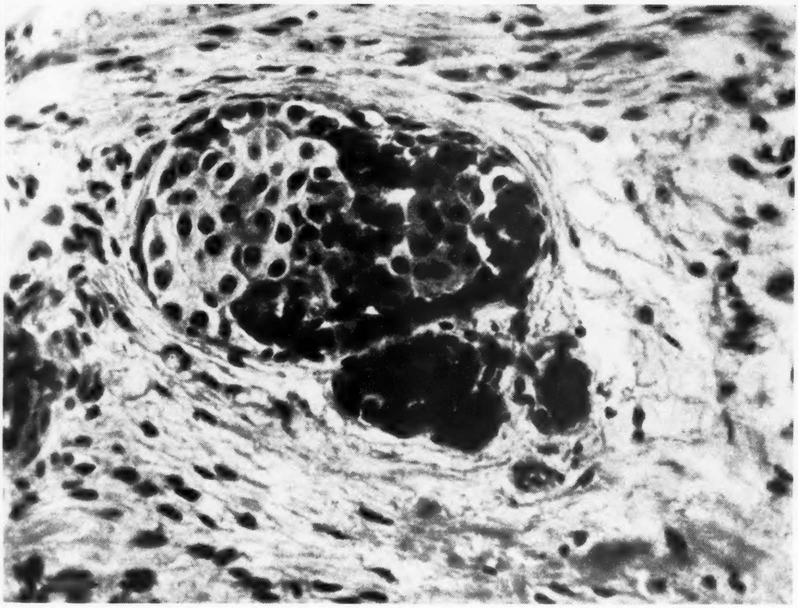

Fig. 46. Pancreas: animal with atrophic exocrine parenchyma after ligation of pancreatic duct (R. 185, group III A). Shows a "compressed" islet surrounded by cirrhotic connective tissue. Low vascularity. Variations in beta cell granulation. Gomori stain. Magnification 500 X.

fairly infrequent and were less vascular than normally, giving a "compact" impression, as if they had been compressed by the cirrhotic connective tissue directly beside the islet tissue. Islets were often seen that gave the impression of the connective tissue having forced its way between the cell cords, dividing up large islets into several smaller ones. Adjacent to the proliferated epithelium of the ducts were numerous small islets, often consisting of only two or three cells. In some places, the appearance was reminiscent of that in the embryonic pancreas. Some examples of the pancreatic structure in these cases are shown in Figs. 46, 47 and 48.

Other Organs

The *lipid content* of the *liver cells* was about the same in group III A as in the control groups. Thus, in the former, the grading was as follows: 5, 4, 6, 5, 2, 5, 5, 4, 3, 5, and in groups IV A and IV D it was 6, 4, 6, 6; 6, 5, 5, 4. The average was thus 4—5 in group III A and 5—6 in the control

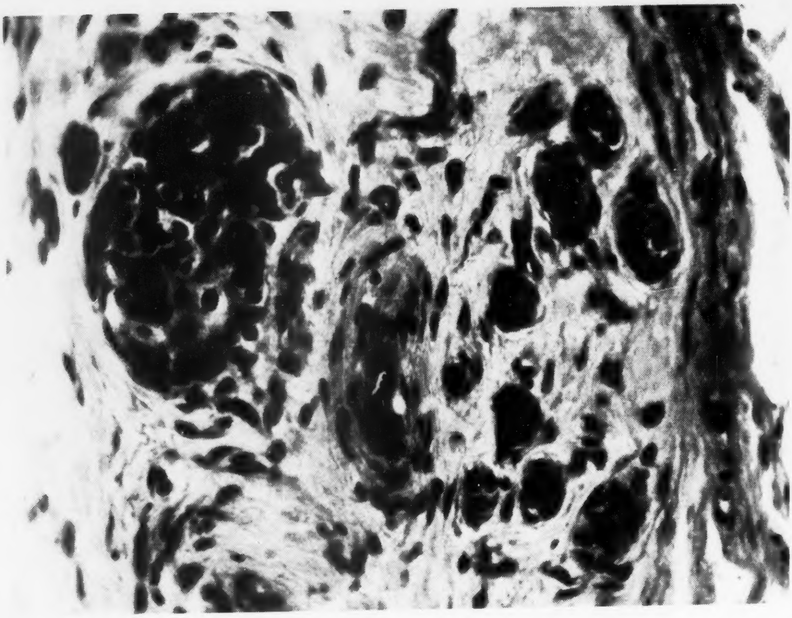

Fig. 47. Pancreas: same animal as in Fig. 46. Several small islets surrounded by connective tissue. Gomori stain. Magnification 500 X.

Fig. 48. Pancreas: same animal as in Fig. 46. Example of suggested new formation of islet cells from duct epithelium. Gomori stain. Magnification 500 X.

groups. The fat droplets had the same general appearance in both groups, *i. e.*, small intracellular droplets which did not interfere with the normal structure of the cells. In no case was any more extensive fatty infiltration observed, with cells entirely filled by fat.

The *glycogen content* of the *liver cells* was graded as follows. Group III A: 7, 7, 6, 7, 7, 7, 7, 7, 7, 7; groups IV A and IV D: 7, 5, 7, 7; 7, 7, 7, 7, the average for all three groups being 6—7.

The *alkaline phosphatase activity* of the *liver cells* was also the same in all the groups; group III A: 7, 6, 6, 6, 6, 6, 6, 5, 6, 6; groups IV A and IV D: 6, 6, 6, 6; 6, 6, 6, 6, *i. e.*, an average of about 6 in all three groups.

The *phosphatase content* of the *duodenal mucosa* and the *lipid content* of the *adrenal cortex* were high and the same in all three groups.

The *phosphatase activity* of both *kidneys* was determined in group IV D. It was present to the normal extent in the right kidney, but only low phosphatase activity was observed in a few remaining epithelial cells of the hydronephrotic left kidney.

B. DIABETIC ANIMALS

Ligation of the pancreatic duct was performed in 5 alloxan-diabetic animals: *group III B.* Four of them were males and 1 female; all were assigned to category 1. The group thus comprised the following animals: R. 201, 202, 217, 218, 215. A brief account of the course and post mortem findings is given in the following animal records.

ANIMAL RECORDS

GROUP III B: CATEGORY 1

R. 201. Male. 1st obs. day 23/7 1954. BW 2.21 kg.—28/7 BW 2.32 kg. *Alloxan:* 100 mg/kg = 230 mg.—29/7 *Alloxan:* same dose as 28/7.—30/7 Massive glycosuria. No acetonuria. Insulin started.—9/8 Condition good. With 16 u insulin (8 regular + 8 NPH) glycosuria 10—15 g/24 hrs. No acetonuria.—10/8 Operation: *Ligation of pancreatic duct.* BW 2.53 kg. Anaesthetic: ether.—12/8 Condition good.—26/8 Condition unchanged. Glycosuria 5—10 g/24 hrs. Insulin: 12 u/24 hrs (6 regular + 6 NPH).—20/9 Condition unchanged. Insulin: 8 u/24 hrs.—10/10 Condition same. Lively, appetite normal. For past few days, glycosuria about 3 g/24 hrs. Insulin: 10 u/24 hrs (6 regular + 4 NPH). BW 3.16 kg. Killed. *Autopsy:* complete atrophy of pancreas; ducts dilated. Otherwise N.A.D. PW 4.35 g (D 2.76 g, L 1.59 g).

R. 202. Male. 1st obs. day 23/7 1954. BW 2.16 kg.—28/7 BW 2.16 kg. *Alloxan:* 100 mg/kg = 215 mg.—29/7 *Alloxan:* same dose as 28/7.—30/7 Massive glycosuria. No acetonuria. Insulin started.—8 Condition good. Glycosuria 10—20 g/24 hrs. Insulin: 12 u/24 hrs (4 regular + 8

NPH).—10/8 Operation: ***Ligation of pancreatic duct.*** BW 2.23 kg. Anaesthetic: ether.—12/8 Condition good. Eats.—20/8 Condition good. Glycosuria 10—20 g/24 hrs. Insulin: 14 u/24 hrs (6 regular + 8 NPH).—10/9 Condition unchanged. Insulin: same dose.—1/10 Glycosuria about 10 g/24 hrs. Insulin 8 u/24 hrs (4 regular + 4 NPH).—13/10 For past week, with 12 u insulin/24 hrs (6 regular + 6 NPH) glycosuria about 10 g/24 hrs. No acetonuria. Condition and appetite good. BW 3.04 kg. Killed. ***Autopsy:*** complete atrophy of pancreas; ducts dilated. Otherwise N.A.D. PW 4.72 g (D 3.12 g, L 1.60 g).

R. 217. Male. 1st obs. day 1/10 1954. BW 2.26 kg.—4/10 BW 2.21 kg. ***Alloxan:*** 100 mg/kg = 220 mg.—5/10 ***Alloxan:*** same dose as 4/10.—6/10 Massive glycosuria. No acetonuria. Insulin started.—17/10 With 6 u insulin/24 hrs (4 regular + 2 NPH) glycosuria less than 10 g/24 hrs. Condition good. No acetonuria.—18/10 Operation: ***Ligation of pancreatic duct.*** BW 2.30 kg. Anaesthetic: ether.—20/10 Condition good. Eats.—30/10 Glycosuria 5—7 g/24 hrs. Insulin: 8 u/24 hrs (4 regular + 4 NPH).—15/11 Glycosuria less than 10 g/24 hrs. Insulin: 4 u NPH/24 hrs.—1/12 Glycosuria unchanged. Insulin 6 u/24 hrs (2 regular + 4 NPH).—16/12 Glycosuria still less than 10 g/24 hrs. No acetonuria. Insulin: 6 u/24 hrs (2 regular + 4 NPH). Condition good. BW 3.15 kg. Killed. ***Autopsy:*** complete atrophy of pancreas; ducts dilated. Otherwise N.A.D. PW 7.86 g (D 4.60 g, L 3.26 g).

R. 218. Male. 1st obs. day 1/10 1954. BW 2.46 kg.—4/10 BW 2.26 kg. ***Alloxan:*** 100 mg/kg = 225 mg.—5/10 ***Alloxan:*** same dose as 4/10.—7/10 Massive glycosuria. No acetonuria. Insulin started.—17/10 Condition good. Insulin: 8 u/24 hrs (4 regular + 4 NPH), glycosuria about 10 g/24 hrs.—18/10 Operation: ***Ligation of pancreatic duct.*** BW 2.35 kg. Anaesthetic: ether.—20/10 Condition good. Eats.—30/10 Glycosuria 10—15 g/24 hrs. Insulin: 10 u/24 hrs (4 regular + 6 NPH).—15/11 Glycosuria and insulin dose unchanged.—30/11 Glycosuria same. Insulin dose raised to 14 u/24 hrs (6 regular + 8 NPH).—16/12 Glycosuria unchanged: 10—15 g/24 hrs. No acetonuria. For past few days, insulin 12 u/24 hrs (6 regular + 6 NPH). Condition and appetite good. BW 3.11 kg. Killed. ***Autopsy:*** complete atrophy of pancreas; ducts dilated. Otherwise N.A.D. PW 5.36 g (D 3.56 g, L 1.80 g).

R. 215. Female. 1st obs. day 1/10 1954. BW 2.36 kg.—4/10 BW 2.39 kg. ***Alloxan:*** 100 mg/kg = 240 mg.—5/10 ***Alloxan:*** same dose as 4/10.—6/10 Massive glycosuria. No acetonuria. Insulin started.—17/10 Insulin: 6 u/24 hrs (4 regular + 2 NPH), glycosuria 3—5 g/24 hrs. Condition good. No acetonuria.—18/10 Operation: ***Ligation of pancreatic duct.*** BW 2.73 kg. Anaesthetic: ether.—20/10 Condition good. Eats.—30/10 Glycosuria 5—10 g/24 hrs. Insulin: 8 u/24 hrs (4 regular + 4 NPH).—11/11 In past 24 hrs, glycosuria less than 5 g. Insulin dose same. In p. m. convulsions; stopped by glucose subcutaneously. Insulin dose reduced to half.—27/11 Glycosuria over 20 g/24 hrs. Insulin again raised to 12 u/24 hrs (6 regular + 6 NPH).—15/12 For past few days, glycosuria 7—15 g/24 hrs. Insulin 8 u/24 hrs (4 regular + 4 NPH). No acetonuria. Condition good. BW 3.40 kg. Killed. ***Autopsy:*** complete atrophy of pancreas; ducts dilated. Otherwise N.A.D. PW 4.62 g (D 2.68 g, L 1.94 g).

ALLOXAN ADMINISTRATION

All five animals were given an alloxan dose of 100 mg/kg of body weight on two successive days; glycosuria appeared immediately afterwards in every case. Two animals had acetonuria for a few days, and four had proteinuria for a few days, but in none of them was the general condition affected concurrently with the onset of diabetes.

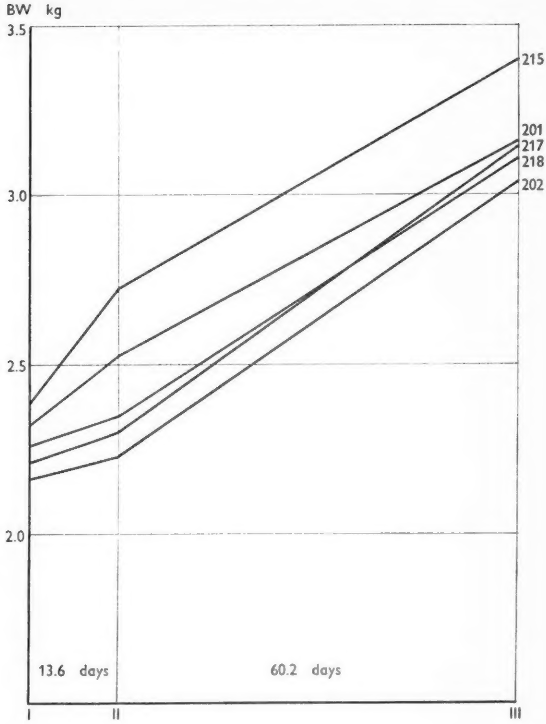

Fig. 49. Change in BW after alloxan administration and after ligation of pancreatic duct: group III B.
I = alloxan administration.
II = operation. III = death.

PRE- AND POSTOPERATIVE COURSE

The diabetic condition was regulated by insulin therapy. Ligation of the pancreatic duct was then performed a mean 13.6 days (13, 13, 14, 14 and 14 days, respectively) after the first alloxan dose.

As in the animals of groups I B and II B, the insulin dose was divided on the day of operation into two smaller doses of regular insulin, and 20 ml of Aminosol-glucose were given subcutaneously after operation. The animals stood the operation well and no complications appeared.

The length of the postoperative observation period was a mean 60.2 days (61, 64, 59, 59 and 58 days).

The pre- and postoperative changes in *body weight* are shown in Fig. 49. There was a normal gain in weight during the entire experimental period, it being a mean 11.7 g/24 hrs before operation, and a mean 12.4 g/24 hrs after it.

TABLE 21

Group III B. Glycosuria and insulin treatment before and after ligation of pancreatic duct

Animal no.	Glycosuria				Insulin treatment			
	Before operation		After operation		Before operation		After operation	
	No. of days	g/24 hrs	No. of days	g/24 hrs	No. of days	Units/24 hrs	No. of days	Units/24 hrs
R. 201	13	12.75	61	9.23	12	9.7	61	9.1
R. 202	13	16.92	64	10.28	12	8.0	64	9.6
R. 217	14	7.41	59	6.63	13	5.7	59	5.2
R. 218	14	6.41	59	10.00	13	5.2	59	9.5
R. 215	14	7.30	58	8.87	13	6.3	58	7.2
$\bar{x}$	14	10.16	60	9.00	13	7.0	60	8.1

DIABETIC CONDITION

As may be inferred from Table 21, the diabetic condition of these animals underwent no essential change during the observation period. Thus, the average urinary glucose was 10 g/24 hrs preoperatively, and 9 g/24 hrs postoperatively. The insulin dose was about 7 u/24 hrs before operation and about 8 u/24 hrs after it.

A comparison can be made with group IV G b, in which the mode of alloxan administration was the same (Table 15). It is then seen that the diabetic condition in group III B was milder with respect to glycosuria and insulin requirement, but that this difference was the same both before and after ligation of the pancreatic duct.

AUTOPSY

Complete atrophy of the pancreas parenchyma was observed in all five cases, the features being the same as in group III A. Microscopic examination also failed to disclose any signs of normally functioning exocrine parenchyma. The mean weight of the tissue dissected out, which subsequently underwent quantitative micromorphologic analysis, was 5.38 g expressed as PW and 1.70 g expressed as PW/BW.

QUANTITATIVE MICROMORPHOLOGIC ANALYSES

Size of Islet Tissue

The results of quantitative determinations of the islet tissue in this group are recorded in Table 25 and Fig. 52. It can be inferred that the calculated islet volume was of the same order of magnitude as in the diabetic groups with functioning exocrine parenchyma. Statistical comparison between group III B and group IV G b showed the following (*cf.* Table 23).

With respect to the number of islets, group III B had a higher mean value than group IV G b. This applied to both n_i and n_i/BW, the difference being significant in both cases. As far as islet size, m_i, is concerned, the mean value in group IV G b was, on the contrary, higher than that in group III B, this difference also being significant. In calculation of the islet volume, this difference in number and size resulted—as could be expected—in no difference being present between the groups, either in V_i or in V_i/BW.

When, on the other hand, group III B was compared with the non-diabetic group without functioning exocrine parenchyma (group III A), no difference was found between them as regards islet number (n_i, n_i/BW). The islet size was, however, significantly lower in group III B, and the islet volume was also smaller than in group III A. The latter difference is probably significant for both V_i and V_i/BW.

Alpha and Beta Cell Count

The mean incidence of alpha cells in group III B was 53.93 per cent, with a range of 47 to 61 per cent. This value is obviously lower than that in group IV G b, in which it was 76.89 per cent. The difference is significant. Compared with the non-diabetic group III A the alpha cell incidence was, however, considerably higher, the difference being highly significant.

HISTOLOGIC AND HISTOCHEMICAL EXAMINATION

Pancreas

As far as the degree of atrophy of the exocrine parenchyma, the quantity of adipose tissue and the infiltration of connective tissue are concerned, the histologic picture was the same in group III B as in group III A. The islet tissue differed from that of the non-diabetic group in those respects confirmed by the quantitative analysis, the islets being fairly numerous, but

all of a strikingly small size. There was an almost complete absence not only of the large islets, but also of those of a more moderate size.

Other Organs

Histochemical examination of the *liver cells* gave the following results. *Lipid content:* 6, 4, 3, 5, 4, *glycogen content:* 7, 7, 7, 7, 7 and *alkaline phosphatase activity:* 6, 5, 6, 6, 6. The average was thus 4—5 for the lipids, 7 for glycogen and 5—6 for phosphatase (*cf.* Table 12).

The *alkaline phosphatase activity* of the *duodenal mucosa* and the *lipid content* of the *adrenal cortex* showed no deviations from other groups, *i. e.*, high phosphatase activity in the duodenum and a large quantity of lipids in all layers of the adrenal cortex.

C. ALLOXAN ADMINISTRATION AFTER LIGATION OF PANCREATIC DUCT

This group consisted of 15 animals, divided into two sub-groups: *group III C a*, in which the effect of alloxan was tested a mean 68.4 days after ligation of the pancreatic duct, and *group III C b*, in which alloxan was administered 11 days after operation. The former group contained 9 animals (2 males and 7 females); all were assigned to category 1. The latter group consisted of 6 animals (2 males and 4 females), all of which were assigned to category 2. Group III C thus comprised the following animals:

Group III C a, category 1: R. 211, 212, 195, 196, 197, 198, 199, 200, 207

Group III C b, category 2: R. 235, 236, 237, 238, 239, 240.

A brief account of the experimental course and the post mortem observations is given in the following animal records.

ANIMAL RECORDS

GROUP III C a: CATEGORY 1

R. 211. Male. 1st obs. day 30/7 1954. BW 2.03 kg.—6/8 Operation: ***Ligation of pancreatic duct.*** BW 2.10 kg. Anaesthetic: ether.—8/8 Condition and appetite good.—15/10 BW 2.30 kg. ***Alloxan:*** 200 mg/kg = 460 mg.—29/10 Hitherto no glycosuria. BW 2.31 kg. ***Alloxan:*** 300 mg/kg = 695 mg.—6/11 Still no glycosuria.—11/11 Still no signs of diabetes. BW 2.34 kg. Killed. ***Autopsy:*** complete atrophy of pancreas; ducts dilated. Otherwise N.A.D. PW 4.70 g (D 3.50 g, L 1.20 g).

R. 212. Male. 1st obs. day 30/7 1954. BW 2.12 kg.—6/8 Operation: ***Ligation of pancreatic duct.*** BW 2.28 kg. Anaesthetic: ether.—4/11 BW 2.68 kg. ***Alloxan:*** 200 mg/kg = 535 mg.—11/11 Hitherto no glycosuria. BW 2.61 kg. ***Alloxan:*** 300 mg/kg = 785 mg.—17/11 Still no signs of diabetes. BW 2.96 kg. Killed. ***Autopsy:*** complete atrophy of pancreas; ducts dilated. Otherwise N.A.D. PW 7.20 g (D 4.20 g, L 3.00 g).

R. 195. Female. 1st obs. day 18/6 1954. BW 1.58 kg.—23/6 Operation: *Ligation of pancreatic duct.* BW 1.65 kg. Anaesthetic: ether.—1/9 BW 2.70 kg. *Alloxan:* 200 mg/kg = 540 mg.—9/9 Hitherto no glycosuria. BW 2.51 kg. *Alloxan:* 300 mg/kg = 755 mg.—21/9 Still no signs of diabetes.—5/10 Glucose tolerance test. BW 2.75 kg. Glucose: 4.4 ml.—17/10 Blood sugar 136 mg/100 ml. BW 2.90 kg. Killed. *Autopsy:* complete atrophy of pancreas; ducts dilated. Otherwise N.A.D. PW 6.29 g. (D 4.35 g, L 1.94 g).

R. 196. Female. 1st obs. day 18/6 1954. BW 1.76 kg.—23/6 Operation: *Ligation of pancreatic duct.* BW 1.78 kg. Anaesthetic: ether.—1/9 BW 2.38 kg. *Alloxan:* 200 mg/kg = 475 mg.—9/9 Hitherto no glycosuria. BW 2.25 kg. *Alloxan:* 300 mg/kg = 675 mg.—21/9 Still no glycosuria.—5/10 Glucose tolerance test. BW 2.06 kg. Glucose: 3.3 ml.—17/10 BW 2.36 kg. Killed. *Autopsy:* complete atrophy of pancreas; ducts dilated. Otherwise N.A.D. PW 3.62 g (D 2.32 g, L 1.30 g).

R. 197. Female. 1st obs. day 19/6 1954. BW 3.02 kg.—21/6 Operation: *Ligation of pancreatic duct.* BW 2.81 kg. Anaesthetic: ether.—20/8 BW 2.86 kg. *Alloxan:* 100 mg/kg = 285 mg.—21/8 *Alloxan:* same dose as 20/8.—26/8 Hitherto no glycosuria. BW 2.98 kg. *Alloxan:* 200 mg/kg = 595 mg.—6/9 Still no glycosuria. BW 3.17 kg. *Alloxan:* 300 mg/kg = 950 mg.—5/10 Glucose tolerance test. BW 3.01 kg. Glucose: 4.8 ml.—6/10 Still no glycosuria. BW 3.24 kg. Killed. *Autopsy:* complete atrophy of pancreas; ducts dilated. Otherwise N.A.D. PW 9.40 g (D 4.46 g, L 4.94 g).

R. 198. Female. 1st obs. day 19/6 1954. BW 2.82 kg.—21/6 Operation: *Ligation of pancreatic duct.* BW 2.80 kg. Anaesthetic: ether.—20/8 BW 2.94 kg. *Alloxan:* 100 mg/kg = 295 mg.—21/8 *Alloxan:* same dose as 20/8.—26/8 Hitherto no glycosuria. BW 3.07 kg. *Alloxan:* 200 mg/kg = 615 mg.—6/9 Still no glycosuria. BW 3.00 kg. *Alloxan:* 300 mg/kg = 900 mg.—5/10 Glucose tolerance test. BW 2.96 kg. Glucose: 4.7 ml.—6/10 Still no glycosuria. BW 3.02 kg. Killed. *Autopsy:* complete atrophy of pancreas; ducts dilated. Otherwise N.A.D. PW 6.26 g (D 4.59 g, L 1.67 g).

R. 199. Female. 1st obs. day 19/6 1954. BW 2.99 kg.—22/6 Operation: *Ligation of pancreatic duct.* BW 2.82 kg. Anaesthetic: ether.—23/8 BW 3.19 kg. *Alloxan:* 200 mg/kg = 640 mg.—31/8 Hitherto no glycosuria. BW 3.17 kg. *Alloxan:* 300 mg/kg = 950 mg.—5/10 Glucose tolerance test. BW 3.15 kg. Glucose: 5.0 ml.—13/10 No glycosuria. BW 3.18 kg. Killed. *Autopsy:* complete atrophy of pancreas; ducts dilated. Otherwise N.A.D. PW 5.71 g (D 3.99 g, L 1.72 g).

R. 200. Female. 1st obs. day 19/6 1954. BW 2.87 kg.—22/6 Operation: *Ligation of pancreatic duct.* BW 2.69 kg. Anaesthetic: ether.—23/8 BW 2.83 kg. *Alloxan:* 200 mg/kg = 565 mg.—31/8 Hitherto no glycosuria. BW 2.93 kg. *Alloxan:* 300 mg/kg = 880 mg.—1/10 Still no glycosuria Glucose tolerance test unsuccessful owing to difficulties in injection. BW 2.77 kg.—13/10 Blood sugar (12 noon, not fasting value) 106 mg/100 ml.—14/10 BW 2.89 kg. Killed. *Autopsy:* complete atrophy of pancreas; ducts dilated. Otherwise N.A.D. PW 6.67 g (D 4.58 g, L 2.09 g).

R. 207. Female. 1st obs. day 30/7 1954. BW 2.05 kg.—3/8 Operation: *Ligation of pancreatic duct.* BW 2.16 kg. Anaesthetic: ether.—15/10 BW 2.93 kg. *Alloxan:* 200 mg/kg = 585 mg.—29/10 Hitherto no glycosuria. BW 2.86 kg. *Alloxan:* 300 mg/kg = 860 mg.—11/11 Still no glycosuria. BW 2.83 kg. Killed. *Autopsy:* complete atrophy of pancreas; ducts dilated. Accessory finding: absence of spleen. Otherwise N.A.D. PW 5.18 g (D 4.12 g, L 1.06 g).

GROUP III C b: CATEGORY 2

R. 235. Male. 1st obs. day 11/1 1955. BW 3.01 kg.—13/1 Operation: *Ligation of pancreatic duct.* BW 2.99 kg. Anaesthetic: ether.—24/1 BW 2.88 kg. *Alloxan:* 100 mg/kg = 290 mg.—25/1 *Alloxan:* same dose as 24/1.—26/1 Massive glycosuria. No acetonuria.—3/2 Continuous glycosuria since 26/1. No acetonuria. Condition good. No insulin given. BW 3.08 kg. Killed. *Autopsy:*

reduction in pancreas parenchyma but not total atrophy. Ducts dilated; increased fat content. Otherwise N.A.D. PW 3.27 g (D 2.05 g, L 1.22 g).

R. 236. Male. 1st obs. day 11/1 1955. BW 2.69 kg.—13/1 Operation: ***Ligation of pancreatic duct*** BW 2.65 kg. Anaesthetic: ether.—24/1 BW 2.45 kg. ***Alloxan:*** 100 mg/kg = 245 mg.—25/1 ***Alloxan:*** same dose as 24/1.—31/1 Hitherto no glycosuria. BW 2.41 kg. ***Alloxan:*** 200 mg/kg = 480 mg.—2/2 Massive glycosuria.—3/2 Glycosuria 7.5 %. No acetonuria. Condition unaffected. BW 2.50 kg. Killed. ***Autopsy:*** pancreas parenchyma still visible macroscopically, although reduced. Ducts dilated; increased fat content. Otherwise N.A.D. PW 2.60 g (D 1.73 g, L 0.87 g).

R. 237. Female. 1st obs. day 11/1 1955. BW 4.51 kg.—14/1 Operation: *Ligation of pancreatic duct.* BW 4.31 kg. Anaesthetic: ether.—25/1 BW 4.12 kg. ***Alloxan:*** 100 mg/kg = 410 mg.—26/1 ***Alloxan:*** same dose as 25/1.—31/1 Hitherto no glycosuria. BW 4.13 kg. ***Alloxan:*** 200 mg/kg = 825 mg.—4/2 Still no glycosuria. BW 3.86 kg. ***Alloxan:*** 300 mg/kg = 1,160 mg.—7/2 Still no glycosuria.—8/2 Glycosuria 1.5 g/24 hrs.—9/2 Glycosuria 3.4 g.—10/2 Glycosuria 5.8 g.—11/2 Glycosuria 9.0 g.—12/2 Glycosuria 18.6 g.—13/2 Glycosuria 26.4 g. BW 4.27 kg. Killed. *Autopsy:* incipient atrophy of pancreas; ducts dilated. High fat content; lobules small. Otherwise N.A.D. PW 6.39 g (D 4.06 g, L 2.33 g).

R. 238. Female. 1st obs. day 11/1 1955. BW 4.52 kg.—14/1 Operation: *Ligation of pancreatic duct.* BW 4.42 kg. Anaesthetic: ether.—25/1 BW 4.06 kg. ***Alloxan:*** 100 mg/kg = 405 mg.—26/1 ***Alloxan:*** same dose as 25/1.—31/1 Hitherto no glycosuria. BW 4.04 kg. ***Alloxan:*** 200 mg/kg = 810 mg.—4/2 Still no glycosuria. BW 4.14 kg. ***Alloxan:*** 300 mg/kg = 1,240 mg.—9/2 Still no glycosuria. Blood sugar 135 mg/100 ml. BW 4.09 kg. Killed. ***Autopsy:*** incipient atrophy of pancreas; ducts dilated. Increased fat content; small lobules. Small infarction in caudal part of left kidney. Otherwise N.A.D. PW 5.88 g (D 3.84 g, L 2.04 g).—***Micr. exam.:*** infarction of left kidney confirmed.

R. 239. Female. 1st obs. day 11/1 1955. BW 3.10 kg. 15/1 Operation: *Ligation of pancreatic duct.* BW 3.14 kg. Anaesthetic: ether.—26/1 BW 3.21 kg. ***Alloxan:*** 100 mg/kg = 320 mg.—27/1 ***Alloxan:*** same dose as 26/1.—31/1 Hitherto no glycosuria. BW 3.27 kg. ***Alloxan:*** 200 mg/kg = 655 mg.—4/2 Still no glycosuria. BW 3.15 kg. ***Alloxan:*** 300 mg/kg = 945 mg.—5/2 No glycosuria.—6/2 Still no glycosuria.—7/2 Glycosuria 2.3 g.—8/2 Glycosuria 2.8 g.—10/2 Glycosuria 5.0 g.—13/2 Glycosuria 7.6 g. Thus, continuous glycosuria since 7/2. BW 3.34 kg. Killed. *Autopsy:* incipient atrophy of pancreas; ducts dilated. High fat content; small lobules. Otherwise N.A.D. PW 7.24 g (D 4.01 g, L 3.23 g).

R. 240. Female. 1st obs. day 11/1 1955. BW 4.70 kg.—15/1 Operation: *Ligation of pancreatic duct.* BW 4.43 kg. Anaesthetic: ether.—26/1 BW 4.23 kg. ***Alloxan:*** 100 mg/kg = 425 mg.—27/1 ***Alloxan:*** same dose as 26/1.—31/1 Hitherto no glycosuria. BW 4.35 kg. ***Alloxan:*** 200 mg/kg = 870 mg.—4/2 Still no glycosuria. BW 4.33 kg. ***Alloxan:*** 300 mg/kg = 1,300 mg.—5/2 Found dead in morning. 1.6 g of glucose in urine voided before death. ***Autopsy:*** BW 4.27 kg. Incipient atrophy of pancreas; ducts dilated. High fat content; lobules small. Large quantity of fluid in pleurae; lungs oedematous. Several small white spots (adipose tissue necroses) in mesenteric adipose tissue.—***Micr. exam.:*** necrotic changes in liver; fatty degeneration centrally in lobules. Extensive necrosis of renal tubules; circumscribed necrotic areas in adrenals.—Probable cause of death: toxic alloxan damage and pulmonary oedema.—Although the alloxan effect could not be definitely evaluated, this animal was considered as diabetic after the last alloxan dose. —Post mortem changes prevented *micr. exam.* of pancreas.

Observation Period

Following ligation of the pancreatic duct, the animals in group III C a were under observation for a mean 68.4 days (70, 90, 69, 69, 60, 61, 62, 62 and 73 days, respectively) before alloxan administration was started. In group III C b, the corresponding period was exactly 11 days in all six cases. After starting alloxan administration, the animals in group III C a were observed for a further mean 39.6 days (27, 13, 46, 46, 47, 47, 51, 52 and 27 days, respectively). The corresponding period in group III C b was a mean 13.7 days (10, 10, 19, 15, 18 and 10 days).

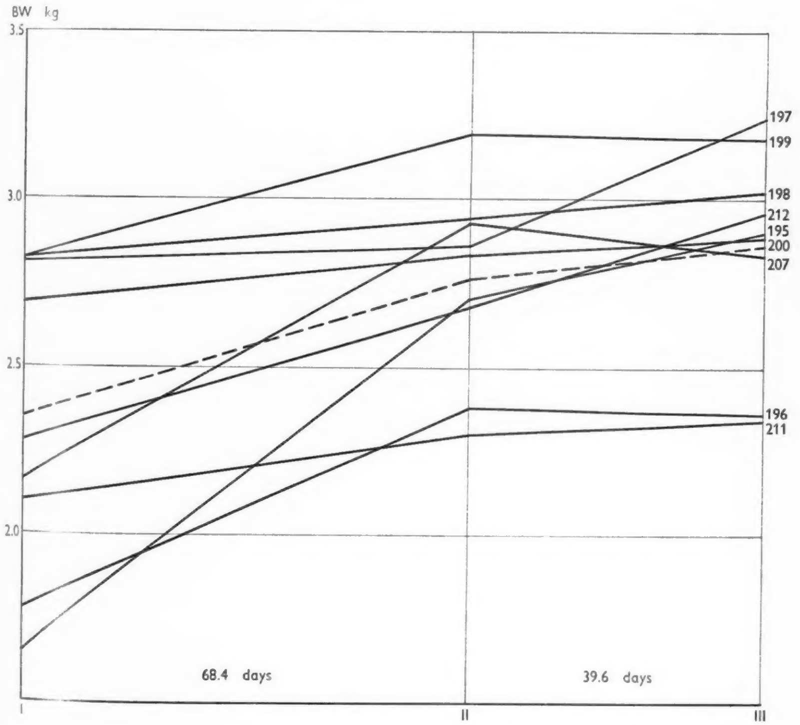

Fig. 50. Change in BW after ligation of pancreatic duct and after subsequent alloxan administration: group III C a.

- - - Mean value. I = operation. II = alloxan administration. III = death.

General Condition

As in groups III A and III B, all the animals in group III C stood the operation well, and no complications arose.

The postoperative changes in *body weight* in group III C a are shown in Fig. 50. Before alloxan administration, all nine animals gained weight, the mean increase being 5.9 g/24 hrs. After alloxan administration, the gain in weight was less marked, and in three cases a loss of weight was recorded (R. 196, 199 and 207). Thus, the mean gain in weight in the second period was 1.6 g/24 hrs in this group.

In group III C b, 5 out of the 6 animals lost weight during the 11 days after operation, so that the mean loss in weight during this time amounted to 15 g/24 hrs. After alloxan administration, these animals gained weight, the mean increase up to the time of death amounting to 7.7 g/24 hrs.

ALLOXAN ADMINISTRATION

Group III C a.—Alloxan was given to these animals in the usual way. In two cases (R. 197 and 198) administration was started with a dose of 100 mg/kg of body weight on two successive days; in the other seven cases, an initial dose of 200 mg/kg was given. When the diabetogenic effect failed to appear, a larger dose (up to 300 mg/kg) was given 9—10 days later. The results are shown in Table 22. In this table, the animals given an initial

TABLE 22

Effect of alloxan administration in normal animals, after ligation of pancreatic duct (group III C), after glucose treatment (group F b), and after ligation of common bile duct (group II C)

Group	No. of animals	No. with diabetes after alloxan dose (mg/kg BW)					No. resistant to alloxan
		100_1	100_2	209	> 200	?	
Normal	a) 16	12	2	0	1	1	0
	b) 19	—	—	17	1	1	0
III C a	a) 2	0	0	0	0	0	2
	b) 7	—	—	0	0	0	7
III C b	a) 6	0	1	1	3	0	1
IV F b	a) 6	1	5	—	—	—	0
II C	a) 2	2	—	—	—	—	0

dose of 100 mg/kg (100_1 and 100_2) are listed under *a*, and those given an initial dose of 200 mg/kg under *b*.

It can be inferred from this table that *all the animals were resistant to the diabetogenic effect of alloxan* even when the highest dose (300 mg/kg) was given. The toxic effects also seemed to be less than when the preparation was given to previously untreated animals. Thus, there were no deaths caused by alloxan damage, even after the highest dose, whereas in previously untreated animals, a dose of 200 mg/kg was associated with a mortality of 6/19, *i. e.*, 31.6 per cent (*cf.* p. 64). Proteinuria of short duration appeared in 4 out of the 9 animals after a dose of 200 mg/kg and in 8 after a dose of 300 mg/kg. In previously untreated animals, proteinuria appeared in every case after a dose of 200 mg/kg. This lower toxicity is, however, probably directly associated with the absence of a diabetogenic effect, since complications more or less closely related to the diabetic condition were among those appearing in the previously untreated animals.

The animals in this group were killed about four weeks after the last dose of alloxan. During this period, they all remained aglycosuric.

Group III C b.—All the animals in this group were given an initial alloxan dose of 100 mg/kg of body weight on two successive days. Glycosuria then appeared in one case. One more animal became diabetic after a dose of 200 mg/kg, and three more after 300 mg/kg. Thus, only one animal (R. 238) was resistant to the diabetogenic effect of alloxan. As may be inferred from Table 22, this result differed from what could have been expected in view of the alloxan effect in the 35 previously untreated animals.

Proteinuria appeared in 3 out of 6 animals after a dose of 100 mg/kg on two successive days, in 1 out of 5 after a dose of 200 mg/kg, and in 3 out of 4 after a dose of 300 mg/kg. After the last-mentioned dose, R. 240 died as a result of toxic, degenerative damage to the liver and kidneys caused by alloxan.

GLUCOSE TOLERANCE TESTS

In five animals in group III C a (R. 195, 196, 197, 198, 199) a glucose tolerance test was made about 41 days after starting alloxan administration, and about 28 days after the last dose had been given. The results are shown in Table I: 9 and Fig. 51.

In these tests, blood samples were taken at longer intervals than in the rest of the material, *i. e.*, at 0, 5, 30, 60 and 90 minutes. This was taken into account in calculation of the tolerance area by changing the time factor

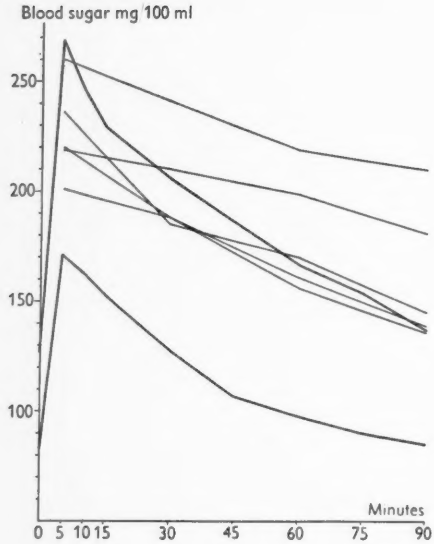

Fig. 51. Glucose tolerance curves: group III C a after operation and after alloxan administration.

to correspond with the longer intervals between the samples. The values obtained are therefore comparable with those in the rest of the material.

The mean value of the tolerance area was 8,584 mg-min. The difference between this value and that in the "normal group" is highly significant both when the *t* test is used and in the WILCOXON test (*cf.* Table 26). Although the type of tolerance curve recorded is of a "diabetic" type as regards its high level and exceedingly slow fall, it should be noted that the fasting value (at time 0) is normal in every case (mean: 103 mg/100 ml; individual values: 99, 100, 103, 103, 109 mg/100 ml).

AUTOPSY

Complete atrophy of the pancreas was observed in group III C a, to the same extent as that described in groups III A and III B. In group III C b, the atrophy was also distinctly visible macroscopically, although larger portions of the glandular parenchyma could be distinguished from the surrounding adipose tissue than in the aforementioned groups. The mean weight of the tissue removed for microscopic examination in group III C a was 6.11 g expressed as PW, and 2.12 g expressed as PW/BW.

Size of Islet Tissue

The results of the analyses of the islet tissue are recorded in Tables 23 and 25 and Fig. 52. It is seen that the calculated islet volume is greater in group III C a than in both the other groups in which the pancreatic duct was ligated. As compared to the diabetic group III B, the difference is

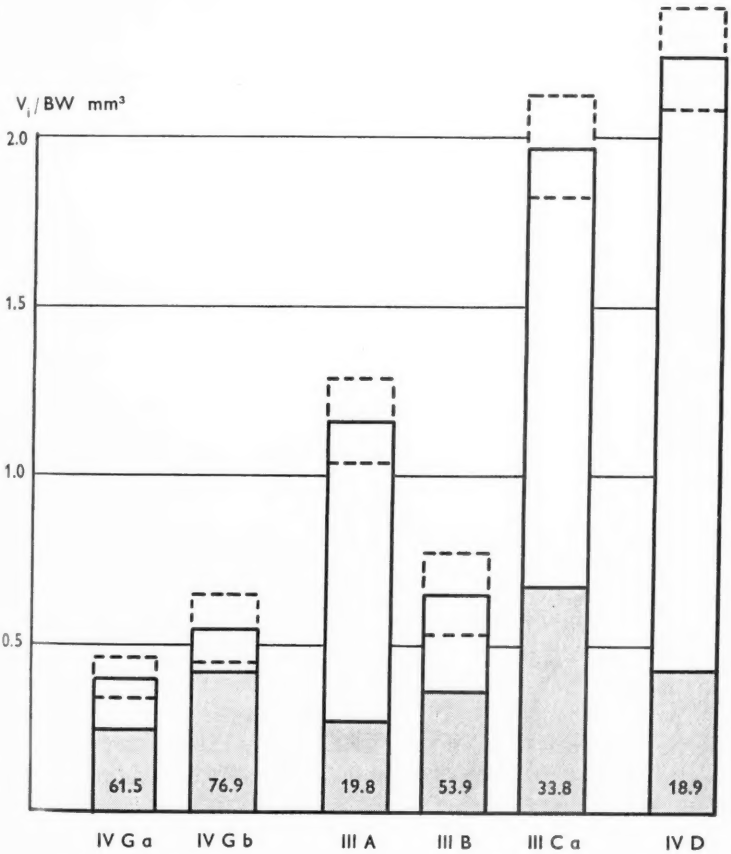

Fig. 52. Islet volume (V_i/BW): comparison between group III (without functioning exocrine parenchyma), group IV G (alloxan-diabetic controls) and group IV D (non-diabetic controls). Mean values — — — $\bar{x} \pm e(x)$. Shaded areas and figures in them denote alpha cell incidence in respective groups.

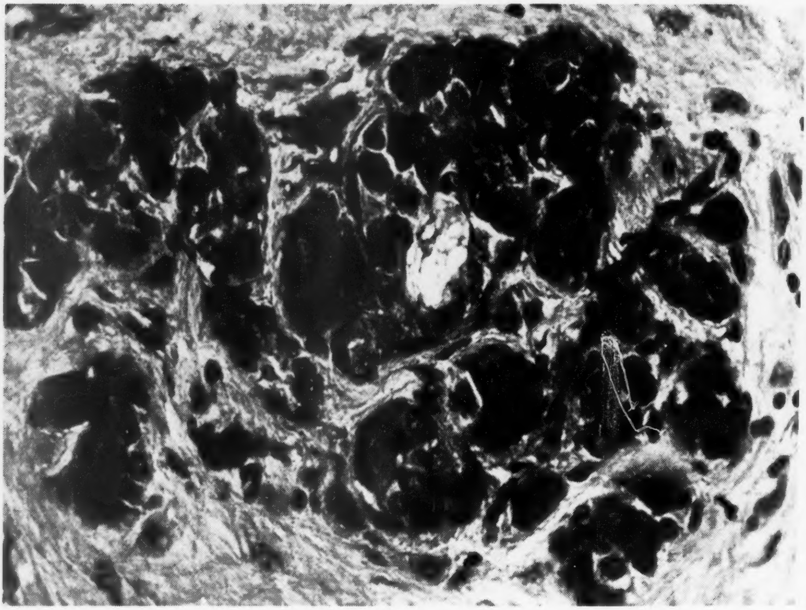

Fig. 53. Pancreas: animal with ligation of pancreatic duct, followed by alloxan administration (R. 198, group III C a). Resistant to diabetogenic effect of alloxan. Exocrine atrophy with cirrhosis. Numerous small islets beside small ducts and duct cells. Predominance of alpha cells. Gomori stain. Magnification about 500 X.

significant or highly significant with respect to all the determinations for evaluation of the islet tissue, *i. e.*, n_i, n_i/BW, m_i, V_i and V_i/BW. But the values are also higher in relation to those in the non-diabetic group III A. The difference is significant with respect to islet number (n_i, n_i/BW) and absolute islet volume (V_i) and probably significant with respect to islet volume per kg of body weight (V_i/BW). Only in the case of islet size (m_i) is there no difference between these two groups (Table 23).

It is also evident from Fig. 52 that the calculated islet volume in group III C a is closer to that in control group IV D (normally functioning exocrine parenchyma) than to that in group III A. Thus, a comparison between groups III C a and IV D showed the mean value for islet number (n_i, n_i/BW) to be higher in the former group, the difference being highly significant, whereas the mean value for islet size was higher in group IV D than in group III C a, this difference also being highly significant.

As a result, no difference is found between the two groups as to V_i and $V_{i/}$BW.

Alpha and Beta Cell Count

The mean alpha cell incidence in group III C a, 33.77 per cent, lies between the corresponding values in groups III A and III B. The scattering in the group was relatively great, the range being 16 to 52 per cent. There was nevertheless a significant difference between the mean and that in both group III A and group III B. In relation to control group IV D, the difference is probably significant (Table 23).

HISTOLOGIC AND HISTOCHEMICAL EXAMINATION

Pancreas

Except for the quantitative differences regarding the islet tissue described in the aforegoing, the general appearance of the pancreatic tissue in group III C a showed the same features as those in group III A (Fig. 53).

Other Organs

Histochemical evaluation of the *liver cells* gave the same results as in groups III A and III B. Thus, the *lipid content* was moderate to fairly high (4, 5, 4, 4, 5, 5, 5, 4, 3), the *glycogen content* was very high (7, 7, 7, 7, 7, 7, 7, 7, 7) and the *alkaline phosphatase content* was also high (6, 6, 6, 6, 6, 6, 4, 5, 6): Table 12.

The *phosphatase activity* in the *duodenal mucosa* and the *lipid content* of the *adrenal cortex* were also the same as in the aforementioned groups. At microscopic examination, foci and strands of connective tissue with a high lymphocyte content were observed in the *kidneys* of 5 animals (R. 197, 198, 199, 200, 207), and in 2 cases (R. 199 and 207) circumscribed areas of tubular necrosis as well. These lesions were interpreted as toxic tissue damage caused by alloxan.

TABLE 23

Statistical comparison between mean size of islet tissue and alpha cell incidence in groups III, IV G and IV D

Groups	df	n_i	n_i/BW	m_i	V_i	V_i/BW	Alpha %
III B —IV G b	8	III B **	III B **	IV G b **	0	0	IV G b **
III B —III A	13	0	0	III A **	III A *	III A *	III B ***
III C a—III B	12	III C a **	III C a ***	III C a ***	III C a ***	III C a ***	III B **
III C a—III A	17	III C a **	III C a **	0	III C a **	III C a *	III C a **
III C a—IV D	11	III C a ***	III C a ***	IV D ***	0	0	III C a *

0 = no difference
* = probably significant difference
** = significant difference
*** = highly significant difference

When a difference is present, the group in which the mean is higher is given in the relevant column.

TABLE 24

Body size in groups with atrophic exocrine parenchyma

		III A	III B	III C a
n		10	5	9
BW kg	$\bar{x}$	2.88	3.17	2.86
	$e(\bar{x})$	0.15	0.06	0.11
	s	0.48	0.14	0.32
BS dm^2	$\bar{x}$	18.1	18.9	18.1
	$e(\bar{x})$	0.39	0.02	0.30
	s	1.24	0.03	0.91

TABLE 25

Quantitative analysis of islet tissue in groups with atrophic exocrine parenchyma

		III A	III B	III C a
n		10	5	9
l	$\bar{x}$	11.3	13.8	14.1
k	$\bar{x}$	5.9	6.4	7.3
N	$\bar{x}$	273	245	327
n_i	$\bar{x}$	2,087.2	1,969.4	3,095.8
	$e(\bar{x})$	148.6	272.9	242.2
	s	469.7	610.1	726.5
n_i/BW	$\bar{x}$	742.3	621.8	1,086.3
	$e(\bar{x})$	66.9	88.9	76.1
	s	211.6	198.8	228.4
m_i μ^2	$\bar{x}$	1,466.9	838.2	1,541.3
	$e(\bar{x})$	133.6	49.0	112.2
	s	422.4	109.5	336.6
V_i mm³	$\bar{x}$	3.70	2.03	5.60
	$e(\bar{x})$	0.43	0.37	0.44
	s	1.37	0.82	1.32
V_i/BW mm³	$\bar{x}$	1.35	0.64	1.97
	$e(\bar{x})$	0.19	0.12	0.15
	s	0.60	0.26	0.46
$\alpha+\beta$	$\bar{x}$	1,358	1,411	1,266
α %	$\bar{x}$	19.82	53.93	33.77
	$e(\bar{x})$	1.63	2.35	3.85
	s	5.15	5.26	11.56

D. DISCUSSION

On the whole, uniform results were obtained in the experiments reported in this chapter, these experiments being directly related to the third and fourth problems listed in the introduction to this paper (p. 13).

Thus, in non-diabetic animals (group III A), ligation of the pancreatic

duct produced a distinct *decrease in size of the islet tissue* in every case, when a micromorphologic analysis of the pancreas was made an average 73 days later. The decrease amounted to 40 to 55 per cent in relation to the controls with functioning exocrine parenchyma. Glycosuria and a change in glucose tolerance appeared in only one case (R. 186), in which the calculated islet volume was the smallest in the group, and the alpha cell incidence the highest. These results are in agreement with earlier experience of the reserve capacity of normal islet tissue. There is reason to recall in particular ALLEN's experiments with subtotal pancreatectomy, showing that diabetic symptoms appear only after removal of seven-eighths to nine-tenths of the pancreatic gland.

The decrease in islet volume was due to a decrease in size of the individual islets, the number of islets remaining unchanged or even being greater than in the controls. Cirrhotic connective tissue invaded the islets, and split them into smaller islets.

I was thus unable to confirm earlier statements that ligation of the pancreatic duct results in hyperplasia of the islet tissue (*cf.* Chapter 1). This discrepancy may be due to species differences. An incomplete quantitative micromorphologic technique may also give misleading results, since only counting of the islets, without measurement of their size, may give a false impression that an increase in the size of the islet tissue has taken place.

The histologic picture in my material was, on the whole, in agreement with that described by BENSLEY in the rabbit after ligation of the pancreatic duct (*cf.* p. 24). The decrease in islet volume indicates that degenerative processes take place. The compressed appearance of the large islets and lack of normal vascularity (*e.g.* Fig. 46) make it probable that the islet tissue degenerates as a result of impaired blood supply. On the other hand, it is not possible to determine, on the basis of the histologic picture alone, whether there is also a new formation of islet tissue from the duct system, as postulated by BENSLEY. This is notwithstanding that the presence of numerous small islets beside the small dilated ducts gives the whole organ a "foetal" appearance, and allows the supposition that new formation of islet tissue does, in fact, occur. It is, however, evident that, at the stage at which the examination was made in the present material, eventual regeneration is unable to compensate for the degenerative, atrophic processes.

Resistance to the diabetogenic effect of alloxan was observed, in complete agreement with the investigation of WALPOLE & INNES (*cf.* p. 26). Resistance was total when alloxan was given about two months after ligation

of the duct (group III C a), and partial when it was given 11 days after operation (group III C b).

Earlier explanations of this resistance have been described in Chapter 1. In the light of the results obtained in the present investigation, the following points can be stressed.

Retention in the gland and passage into the blood stream of *pancreatic enzymes* after ligation of the duct cannot be responsible for the resistance to alloxan. Diastasuria was found to reach a maximum already during the first two days after operation (Table 19), at which time alloxan resistance is stated by WALPOLE & INNES to be lacking. It is, on the contrary, apparent that cessation of enzyme production in the acinar tissue is a prerequisite for alloxan resistance.

ADAMS' hypothesis that the alloxan in the fibrotic pancreas is unable to reach the islets because of the impaired blood supply seems to be less probable. Even if the blood supply of the islets is decreased, it is obviously not entirely cut off, thus preventing alloxan from exerting its diabetogenic effect. It is unlikely that such a large dose as 300 mg per kg of body weight would be unable, on the grounds of an impaired blood supply, to damage islet tissue that is already quantitatively reduced by ligation of the duct.

DE MOOR's theory, that alloxan resistance is due to islet hyperplasia caused by duct ligation, cannot be applied in the rabbit since, as shown by my investigations, no such hyperplasia occurs in this animal. But even if this were the case, the theory appears improbable in view of the results obtained in my groups II C and IV F b, in which animals were found to be normally sensitive to alloxan, despite the fact that they had undergone interventions shown to produce an increase in the size of the islet tissue.

Another hypothesis inapplicable to the rabbit is that of FERNER (*cf.* p. 28). No accumulation of excess fat in the liver was observed in any of the 33 animals in my material in which ligation of the pancreatic duct had been successful. The average lipid content of the liver cells was the same as in the controls (Table 12). When fat was present, it was invariably in the form of intracellular droplets, which in no way altered the general appearance of the liver cells. In most of the cases, the glycogen content of the liver cells was high. The normal glucose tolerance tests in group III A (with the exception of R. 186) also argue against the hypothesis that the liver has lost its ability to regulate the blood sugar in this condition. Nor was any progressive "improvement" in the diabetic condition noted in group III B, in which the animals were alloxan-diabetic before ligation of the pancreatic duct. It is thus evident that no gross fatty infiltration of the liver, with disturbances in its normal functions, occurs two to three

months after ligation of the pancreatic duct in the rabbit, as has been described in the dog.

A further indication of the improbability of FERNER's theory is given by the results of the quantitative analysis of the islet tissue in group III C a. The values were not only higher for islet number, size and volume than in the diabetic group III B—the differences being significant or highly significant—but they were also higher for islet number and volume than in the non-diabetic group III A (Table 23, Fig. 52). Compared with control group IV D with functioning exocrine parenchyma, the islet volume in group III C a was of the same order of magnitude, the islet number being greater but the islet size smaller than in the former group. The atrophy of the islet tissue observed in the alloxan-diabetic animals was thus lacking in group III C a.

In two probably related respects there were, however, indications that alloxan had to some extent affected the islet tissue of the animals in group III C a, *i.e.*, as to glucose tolerance and alpha cell incidence. All five animals in this group, in which glucose tolerance tests were made after alloxan administration, showed signs of decreased tolerance, and an increased tolerance area, which differed significantly from that in the "normal group". SHIPLEY & RANNEFELD obtained similar results in previously untreated rats, given 14 subdiabetogenic doses of alloxan in the course of four weeks. The same experience was made by MOLANDER & KIRSCHBAUM, also in the rat, under analogous conditions. They concluded that, under certain conditions, small repeated doses of alloxan can lead to a functional change in the beta cells although, microscopically, they appear to be intact.

The average alpha cell incidence was higher in my group III C a than in the non-diabetic groups III A and IV D, but lower than in the definitely diabetic group III B. This fact as well indicates that some damage to the beta cells had occurred, although it was by no means comparable to that produced by alloxan in intact animals.

The reason why the islet volume in group III C a was greater than in both other pancreas-ligated groups is uncertain. A conceivable explanation is that the irritation of the islets implied by the repeated doses of alloxan also stimulated the islet cells to reparative and regenerative processes, which led to an increase in the size of the islet tissue. In this connexion, mention can be made of the transient stimulating effect of alloxan on the beta cells observed by FALLER and by ARNOLD.

Another and perhaps more likely explanation is that the postoperative observation period was longer in group III C a (about 108 days) than in

group III A (average 73 days) and group III B (average 60 days). According to BENSLEY, a *progressive* increase in the islet tissue by new formation of islets takes place in the rabbit pancreas after ligation of the duct (*cf.* p. 25). If this statement is correct, it seems natural for the islet volume to be greater in animals that have survived for a longer period after operation than in those killed earlier in the postoperative period.

BENSLEY's view also lends support to the hypothesis of CAPPELLATO & PERISSONOTTO, *i.e.*, that resistance to the diabetogenic effect of alloxan may be associated with the foetal nature of the islet tissue after duct ligation. Additional evidence has been given by other investigations. Thus, animals *in utero* and newborn animals are resistant to alloxan (*cf.* DE MOOR). SHULTZ & DUKE have shown that, up to nine days of age, rabbits are resistant even to large doses of the substance. Furthermore, in a study of the rat pancreas after prolonged administration of small doses of alloxan, HUGHES & HUGHES found indications that young beta cells are more resistant to this substance than are older ones.

Mention may also be made of the investigation by HULTQUIST & THORELL. They found that cells from the foetal pancreas, which with ordinary granule-staining techniques exhibited alpha cell properties, showed other features than alpha cells from adults when they were studied by ultraviolet microscopy. The authors concluded that "the cells during foetal development which display staining properties of the same type as the alpha or silver cells cannot be compared, from the functional point of view, with alpha or silver cells of the adult type". Analogous functional differences might be the explanation of alloxan resistance in foetuses and in newborn animals, as well as in the duct-ligated animals with islet tissue of a foetal nature. The absence of active secretion from the acinar parenchyma may be an important factor in all these cases.

E. SUMMARY

Ligation of the pancreatic duct was performed on 34 animals. In 4 of them, the experiment could not be completed. Of the remaining 30 animals, 10 were non-diabetic (group III A). Five animals were alloxan-diabetic before operation (group III B), and in 15 the effect of alloxan was tested after ligation, either about 68 days later (group III C a) or 11 days later (group III C b).

The *controls* consisted of 8 animals; 4 of them were untreated (group IV A), and in 4 the left ureter was ligated (group IV D). The alloxan-

diabetic controls described in Chapter 6 (group IV G b) served as additional controls.

An account of the *course* is given. The general condition of the animals was unaffected, and a postoperative nutritional disturbance was observed in one case only. A gain in weight was recorded in most of the other cases.

Transient *diastasuria* appeared postoperatively. It was maximal during the first 2 days after operation, but disappeared 4 to 6 days after it (Table 19).

A *glucose tolerance test* was made postoperatively in 9 animals in group III A; in 8 of them it was entirely within the normal range of variation. In the remaining animal, a completely abnormal curve of "diabetic" type was recorded. In contrast to the others in this group, this animal had transient glycosuria postoperatively (Table I: 8).

Glucose tolerance tests made in 5 animals in group III C a after alloxan administration showed signs of decreased tolerance in every case (Table I: 9, Fig. 51).

The *diabetic condition* of the animals in group III B showed no changes as to glycosuria and insulin requirement after ligation of the duct (Table 21).

Complete *resistance to the diabetogenic effect of alloxan* given in large repeated doses was noted in group III C a, and partial resistance in group III C b (Table 22).

Autopsy showed complete *exocrine atrophy of the pancreas* in all the animals in groups III A, III B and III C a, with one exception. In this case (in group III A) an extremely small remains of the gland was present in the pyloric region. In group III C b, the exocrine parenchyma exhibited marked *regressive changes,* although there was not yet complete atrophy.

Quantitative analysis of the *islet tissue* showed a decrease in islet volume of 40 to 55 per cent in group III A in relation to the controls, but a normal alpha cell incidence. In group III B, the islet volume was of the same order of magnitude as in the non-operated alloxan-diabetic controls. The alpha cell incidence was much higher than normally, but not fully as high as in the diabetic controls. In group III C a, the islet volume was distinctly greater than in both preceding groups, and as large as in control group IV D. The alpha cell incidence in group III C a was higher than in the other non-diabetic animals, but lower than in the diabetic animals in group III B (Table 23).

In all the duct-ligated animals, the individual islet size was smaller but the number of islets greater than in comparable controls with functioning exocrine parenchyma.

In *no case* were any signs observed of *damage to the liver parenchyma.*

The lipid and glycogen content of the liver cells was the same as in the controls (Table 12).

The results are discussed. They are, on the whole, in agreement with those obtained by BENSLEY, indicating that, in the rabbit pancreas, both regressive and progressive processes in the islet tissue occur after ligation of the pancreatic duct. It was not possible to confirm earlier statements of hyperplasia of the islet tissue after this intervention.

The alloxan resistance of duct-ligated animals also confirms earlier investigations (WALPOLE & INNES). It cannot be explained by supposed islet tissue hyperplasia (DE MOOR), by impairment of the blood supply (ADAMS) or by postulated liver damage, masking diabetes (FERNER).

In my opinion, the alloxan resistance is dependent on the "foetal" nature of the islet tissue after ligation of the pancreatic duct (CAPPELLATO & PERISSONOTTO), the absence of active external secretion possibly being an important factor. Support is lent to this view by the results of similar investigations (HUGHES & HUGHES, HULTQUIST & THORELL, SHULTZ & DUKE).

CHAPTER 8

GLUCOSE ADMINISTRATION

In some of the investigations mentioned in Chapter 1, administration of glucose to experimental animals was found, under certain conditions, to lead to hyperplasia of the pancreatic islet tissue. Against this background, I considered it of interest to include in the present investigation some experiments with parenteral administration of glucose. If it could be confirmed quantitatively that enlargement of the islet tissue actually took place, the degree of this enlargement could be compared with that found to result from interruption of the normal bile flow. In addition, I wished to ascertain whether the diabetogenic effect of alloxan underwent any change when it was administered to animals that had previously been given glucose for a certain period.

The group given glucose comprised 12 animals, divided into two subgroups. *Group IV F a* consisted of 6 animals (4 males and 2 females) in which the effect of glucose only was investigated, and *group IV F b* of 6 animals (3 males and 3 females) in which the effect of alloxan after glucose administration was studied. All the animals in the former group were assigned to category 1, whereas all those in the latter were assigned to category 2, since the main interest was focused on the alloxan effect, and not on the quantitative conditions with respect to the islet tissue. These groups thus consisted of the following animals:

Group IV F a, category 1: R. 225, 226, 227, 228, 229, 230

Group IV F b, category 2: R. 249, 250, 251, 252, 253, 254.

A brief account of the course and the post-mortem observations is given in the animal records which follow.

ANIMAL RECORDS

GROUP IV F a: CATEGORY 1

R. 225. Male. 1st obs. day 22/10 1954. BW 2.83 kg.—26/10 Glucose tolerance test. BW 2.71 kg. Glucose: 4.3 ml.—2/11 *Glucose administration* started: 22 ml of 30 % glucose subcutaneously twice daily (13.2 g glucose/24 hrs); Sundays once only.—2/12 Glucose tolerance test. BW 2.70 g. Glucose: 4.3 ml.—6/12 Altogether 409.2 g of glucose given in 35 days. Condition unaffected.

BW 2.73 kg. Killed. *Autopsy:* slightly increased vascularity and mild oedema at site of injections. Otherwise N.A.D. PW 3.11 g (D 2.17 g, L 0.94 g).

R. 226. Male. 1st obs. day 22/10 1954. BW 2.24 kg.—26/10 Glucose tolerance test. BW 2.25 kg. Glucose: 3.6 ml.—2/11 *Glucose administration* started (same as in R. 225).—2/12 Glucose tolerance test. BW 2.45 kg. Glucose: 3.9 ml.—6/12 Altogether 409.2 g of glucose given in 35 days. Condition unaffected. BW 2.72 kg. Killed. *Autopsy:* slightly increased vascularity at site of injections. Otherwise N.A.D. PW 3.50 g (D 2.40 g, L 1.10 g).

R. 227. Male. 1st obs. day 22/10 1954. BW 2.62 kg.—28/10 Glucose tolerance test. BW 2.56 kg. Glucose: 4.1 ml.—2/11 *Glucose administration* started (same as in R. 225).—3/12 Glucose tolerance test. BW 3.05 kg. Glucose: 4.9 ml.—7/12 Altogether 422.4 g of glucose given in 36 days. Condition unaffected. BW 3.17 kg. Killed. *Autopsy:* Slightly increased vascularity at site of injections. Several small white spots on surface and cut surface of kidneys. Otherwise N.A.D. PW 4.82 g (D 2.86 g, L 1.96 g).—*Micr. exam.:* interstitial infiltration of lymphocytes in kidneys, corresponding to changes seen macroscopically; no necrosis, no giant cells.

R. 228. Male. 1st obs. day 22/10 1954. BW 2.44 kg.—28/10 Glucose tolerance test. BW 2.31 kg. Glucose: 3.7 ml.—2/11 *Glucose administration* started (same as in R. 225).—3/12 Glucose tolerance test. BW 2.73 kg. Glucose: 4.4 ml.—7/12 Altogether 422.4 g of glucose given in 36 days. Condition unaffected. BW 3.00 kg. Killed. *Autopsy:* N.A.D. PW 4.33 g (D 2.86 g, L 1.47 g).

R. 229. Female. 1st obs. day 2/11 1954. BW 2.77 kg.—5/11 Glucose tolerance test. BW 2.54 kg. Glucose: 4.1 ml.—7/11 *Glucose administration* started (same as in R. 225).—8/12 Glucose tolerance test. BW 2.89 kg. Glucose: 4.6 ml.—9/12 Altogether 382.8 g of glucose given in 33 days. Condition unaffected. BW 3.13 kg. Killed. *Autopsy:* somewhat increased vascularity and slight oedema at site of injections. Otherwise N.A.D. PW 3.78 g (D 2.16 g, L 1.62 g).

R. 230. Female. 1st obs. day 2/11 1954. BW 2.37 kg.—5/11 Glucose tolerance test. BW 2.20 kg. Glucose: 3.5 ml.—7/11 *Glucose administration* started (same as in R. 225).—8/12 Glucose tolerance test. BW 2.54 kg. Glucose: 4.1 ml.—9/12 Altogether 382.8 g of glucose given in 33 days. Condition unaffected. BW 2.75 kg. Killed. *Autopsy:* slightly increased vascularity at site of injections. Otherwise N.A.D. PW 3.19 g (D 2.14 g, L 1.05 g).

GROUP IV F b: CATEGORY 2

R. 249. Female. 1st obs. day 11/2 1955. BW 3.17 kg.—14/2 *Glucose administration* started: 22 ml of 30 % glucose subcutaneously twice daily.—14/3 Altogether 376.2 g of glucose given. Condition unaffected. Glucose discontinued.—15/3 BW 3.29 kg. *Alloxan:* 100 mg/kg = 330 mg. —16/3 *Alloxan:* same dose as 15/3.—17/3 Massive glycosuria.—5/4 Continuous glycosuria since 17/3; average 19.8 g/24 hrs. No acetonuria. BW 3.47 kg. Killed. *Autopsy:* N.A.D. PW 5.27 g (D 3.37 g, L 1.90 g).

R. 250. Female. 1st obs. day 11/2 1955. BW 3.87 kg.—14/2 *Glucose administration* started (same as in R. 249).—14/3 Altogether 376.2 g of glucose given. Condition unaffected. Glucose discontinued.—15/3 BW 3.83 kg. *Alloxan:* 100 mg/kg = 385 mg.—16/3 *Alloxan:* same dose as 15/3.—17/3 Massive glycosuria.—3/4 Continuous glycosuria since 17/3; average 26.1 g/24 hrs. No acetonuria. BW 3.92 kg. Killed. *Autopsy:* several small white spots on surface of kidneys, corresponding to white streaks from capsule running towards borderline between cortex and medulla on cut surface. Otherwise N.A.D. PW 4.04 g (D 2.14 g, L 1.90 g).—*Micr. exam.:* interstitial infiltration of lymphocytes in kidneys corresponding to changes seen macroscopically.

R. 251. Female. 1st obs. day 11/2 1955. BW 3.37 kg.—14/2 *Glucose administration* started (same as in R. 249).—14/3 Altogether 376.2 g of glucose given. Condition unaffected. Glucose discontinued.—15/3 BW 3.53 kg. *Alloxan:* 100 mg/kg = 355 mg.—16/3 *Alloxan:* same dose as 15/3.—17/3 Massive glycosuria; acetonuria. 19/3 For past 24 hrs increasing lethargy, somewhat forced

respiration, massive acetonuria. Died during day, presenting picture of diabetic acidosis. *Autopsy:* BW 3.01 kg. Considerable lipaemia. Serum cholesterol 850 mg/100 ml. Other organs: N.A.D. PW 5.49 g (D 3.26 g, L 2.23 g).—*Micr. exam.:* severe fatty degeneration of liver cells, total at some sites. Kidneys: tubular necrosis.

R. 252. Male. 1st obs. day 11/2 1955. BW 3.05 kg.—21/2 *Glucose administration* started (same as in R. 249).—20/3 Altogether 343.2 g of glucose given. Condition unaffected. Glucose discontinued.—22/3 BW 3.12 kg. *Alloxan:* 100 mg/kg = 310 mg.—23/3 *Alloxan:* same dose as 22/3.—25/3 Massive glycosuria.—5/4 Continuous glycosuria since 25/3; average 35.8 g/24 hrs. No acetonuria. BW 3.18 kg. Killed. *Autopsy:* N.A.D. No lipaemia. PW 3.27 g (D 2.02 g, L 1.25 g).

R. 253. Male. 1st obs. day 11/2 1955. BW 3.10 kg.—21/2 *Glucose administration* started (same as in R. 249).—20/3 Altogether 343.2 g of glucose given. Condition unaffected. Glucose discontinued.—22/3 BW 3.35 kg. *Alloxan:* 100 mg/kg = 335 mg.—23/3 *Alloxan:* same dose as 22/3.—24/3 Massive glycosuria.—4/4 Continuous glycosuria since 24/3; average 11.5 g/24 hrs. No acetonuria. BW 3.51 kg. Killed. *Autopsy:* N.A.D. PW 5.37 g (D 3.61 g, L 1.76 g).

R. 254. Male. 1st obs. day 11/2 1955. BW 3.34 kg.—21/2 *Glucose administration* started (same as in R. 249).—20/3 Altogether 343.2 g of glucose given. Condition unaffected. Glucose discontinued.—22/3 BW 3.29 kg. *Alloxan:* 100 mg/kg = 330 mg.—23/3 *Alloxan:* same dose as 22/3.—25/3 Massive glycosuria.—4/4 Continuous glycosuria since 25/3; average 8.3 g/24 hrs. No acetonuria. BW 3.69 kg. Killed. *Autopsy:* N.A.D. PW 4.22 g (D 2.85 g, L 1.37 g).

COURSE

Glucose Administration

The dosage was 22 ml of a 30 per cent glucose solution twice daily, injected subcutaneously into the back. In group IV F a, the administration was continued for 33 to 36 days (mean: 34.7 days) and in group IV F b for 28 to 29 days (mean: 28.5 days). The total mean quantity of glucose administered amounted to 404.8 g in group IV F a, and to 359.7 g in group IV F b. Thus, the animals in the latter group were given glucose for a somewhat shorter time and received a smaller total quantity than those in the former group.

General Condition

In group IV F a, the general condition of the animals was entirely unaffected during the whole experimental period. No glycosuria appeared. In group IV F b, the appetite was slightly impaired and glycosuria was occasionally present in two of the animals (R. 253 and 254) but did not exceed 5 g/24 hrs.

This difference between the two groups was also reflected in the *body weight.* In group IV F a, all the animals gained weight during the time of glucose administration, the mean gain being 13.1 g/24 hrs. In group

IV F b, three animals lost weight during the corresponding period (R. 250, 252 and 254), the mean change in weight for the whole group being + 1.80 g/24 hrs.

At the beginning of the experiments, the mean weight of the animals in group IV F a was 2.55 kg, and that in group IV F b was 3.32 kg, the animals in the former group being somewhat younger. It is therefore possible that they were better able to withstand the stress of prolonged glucose administration, and thus were less affected than the animals in group IV F b.

GLUCOSE TOLERANCE TESTS

In group IV F a, a glucose tolerance test was made both before and after the period of glucose administration. The results are recorded in Tables I: 11—12. The mean value of the tolerance area was 5,489 mg-min before the experiment and 4,713 mg-min after it. Both these values lie entirely within the normal range (*cf.* Table 26). In a comparison of the differences between the tests in the individual animals, d = —775.8 mg-min was obtained. Evaluated with the *t* test, $t = 1.848$, df = 5 and $P \sim 0.1$. Thus, although a tendency to increased glucose tolerance was present after administration, the difference is not significant.

ALLOXAN ADMINISTRATION

After glucose administration had been discontinued, the animals in group IV F b were given a dose of 100 mg/kg of alloxan on two successive days. This produced diabetes in every case (Table 22). One (R. 251) died 4 days after the second dose, presenting a picture of diabetic acidosis. Another animal had acetonuria, which did not affect its general condition, and two had proteinuria. The animals were killed an average 16 days after alloxan administration; all of them had exhibited continuous glycosuria until their death.

AUTOPSY

Slightly increased vascularity was usually seen at the site of the glucose injections. Macroscopically, the pancreas was normal. In group IV F a, the mean PW was 3.79 g and the mean PW/BW was 1.29 g. In group IV F b, the corresponding values were 4.61 g and 1.35 g. Thus, these groups did not differ as to pancreas weight from controls not given glucose (Table 10).

Some interstitial infiltration of lymphocytes in the kidneys was observed in R. 227 and R. 250. In R. 251 (the animal that died of diabetic acidosis), there was considerable lipaemia, and microscopic examination showed severe fatty degeneration of the liver cells, as well as necrosis of the renal tubules.

QUANTITATIVE MICROMORPHOLOGIC ANALYSES

Size of Islet Tissue

The results of calculations of the islet tissue size in group IV F a are shown in Table 11 and Fig. 34 in Chapter 5 (pp. 134 and 129). It can be inferred that the means for both islet number and islet size, as well as for the islet volume calculated from them, are greater than the corresponding means in any other group in the material. The scattering in the group was, however, considerable.

As an example, the values of V_i can be mentioned; they ranged from a minimum of 6.33 mm³ to a maximum of 25.88 mm³. The latter excessively high figure (R. 225) was the highest value for islet volume obtained in the whole investigation. It is evident from these figures that whereas some animals reacted to glucose administration with considerable enlargement of the islet tissue, others did not react in this respect.

The following results were obtained in a statistical comparison with the entirely untreated control group IV A. Islet number (n_i, n_i/BW): probably significant difference ($P \sim 0.05$). Islet size (m_i): no significant difference ($P > 0.9$). Islet volume (V_i): no significant difference ($P \sim 0.2$); V_i/BW: no significant difference ($P \sim 0.1$).

If the trauma implied by the daily glucose injection is regarded as equivalent to that of ureteric ligation, a comparison between group IV F a and group IV D might be justified. Such a comparison shows a greater islet volume (V_i/BW) in the former group, the difference being probably significant. The difference is due to a greater number of islets in group IV F a.

Comparison between group IV F a and the two groups in which bile flow interventions were made (groups I A and II A) showed no significant difference with respect to size of the islet tissue.

Alpha and Beta Cell Count

The alpha cell incidence was normal in this group, the mean value being 16.14 per cent. In one animal (R. 225, in which the great increase

in islet volume was noted) the incidence was low, *i. e.*, 7.64 per cent; in the others, it ranged from 13 to 24 per cent.

HISTOLOGIC AND HISTOCHEMICAL EXAMINATION

Pancreas

The general appearance of the pancreas in the animals of category 1 exhibited no abnormal features, apart from the quantitative changes in the islet tissue already described.

In one animal (R. 229) islet haemorrhage of the same type as that described in the bile flow experiments was observed, although it was less severe.

Other Organs

The following histochemical grading was made of the *liver cells. Lipids:* 6, 6, 5, 4, 5, 3; *glycogen* 6, 6, 6, 6, 7, 6; *alkaline phosphatase:* 6, 6, 6, 6, 6, 6. Thus, the average was 4—5 for the lipids, 6—7 for glycogen, and 6 for phosphatase. The results did not differ from the findings in the controls (*cf.* Table 12).

Determinations of the *phosphatase activity* in the *duodenum* and the *lipid content* of the *adrenal cortex* also showed normal conditions.

SUMMARY

Six animals were given a moderate dose of glucose subcutaneously twice daily for about four weeks. In some of them, a considerable increase in the islet tissue was observed; in others, there was a slight increase only or none at all. Owing to this variation in the response, no significant difference was found between the size of the islet tissue in this group and that in a group of controls not given glucose, although the mean value of the islet volume was considerably higher in the former group. Nor was there any significant difference in this respect in comparison with the mean values in the bile flow experiment groups.

The mean incidence of alpha cells in the pancreas was found to be within the normal range.

No significant difference was found between the glucose tolerance before and after glucose administration, even in those animals in which the islet volume was greatly increased.

Another six animals were given alloxan after four weeks' administration of glucose. The diabetogenic effect of alloxan was found to be the same as in previously untreated animals.

CHAPTER 9

GENERAL DISCUSSION

GLUCOSE TOLERANCE

Many factors influence the changes in blood sugar after glucose administration. In an intravenous tolerance test, it is the rate of removal of the excess of blood glucose which determines the appearance of the curve (AMATUZIO *et al.*), *i.e.*, a combined diffusion and assimilation phenomenon (CONARD *et al.*).

Some factors of special importance in evaluation of the glucose tolerance are discussed in MARBLE's survey. The role of the previous diet is known from the investigations of HIMSWORTH and of SWEENEY. The glucose tolerance increases with a rising carbohydrate content in the diet, according to the former author even irrespective of its total caloric content, although SWEENEY found that starvation results in the same low glucose tolerance as a low-carbohydrate diet. The Staub-Traugott phenomenon, which is related to this diet factor, has been thoroughly discussed in SOMERSALO's monograph, among other publications.

Other factors mentioned by MARBLE are chiefly of clinical interest, *e.g.* infections, previous insulin administration, age, physical inactivity and endocrine disorders.

According to SWEENEY, the normal tolerance curve is an expression of stimulation of the pancreas by glucose to an increased insulin output, the return of the blood sugar to the fasting level being a direct result of this increased insulin effect. The accuracy of this conception must, however, be questioned in the light of more recent knowledge of the complex nature of the blood-sugar regulating mechanisms (*cf.* MCQUARRIE). The investigations of Soskin and his co-workers in particular have contributed to elucidating the role of the liver in the glucose tolerance test (see SOSKIN & LEVINE). They showed that hepatectomized dogs, given glucose continuously in sufficient doses to keep the blood sugar on the normal level, exhibited markedly "diabetic" curves, whereas entirely normal curves were recorded in pancreatectomized dogs given constant intravenous

injections of insulin. They therefore concluded that, in normal animals, the appearance of the glucose tolerance curve does not depend on an additional secretion of insulin from the pancreas, but is due to hepatic regulatory factors.

In the opinion of SOSKIN & LEVINE, the hepatic blood-sugar regulating mechanisms are analogous to the thermostat-furnace arrangement for temperature regulation of modern buildings, the endocrine balance between the anterior pituitary and the pancreas determining the setting of the thermostat.

However, MACKLER *et al.* have shown that the continuous decrease in blood sugar produced by hepatectomy in the dog is accelerated if glucose is injected concurrently, but that this acceleration is lacking in eviscerated dogs. This is interpreted by the authors as an indication that glucose stimulates to increased insulin secretion.

Consequently, it appears probable that the appearance of the glucose tolerance curve is dependent on both hepatic and pancreatic factors, among others.

The results of the glucose tolerance tests in the present material are assembled in Table 26. It can be inferred that a deviation from the tolerance area in the "normal group" was present in three groups only, *i.e.*, groups II A, III C a and IV E, in which the tolerance was lower. Group II A was ruled out on evaluation with WILCOXON's test (see Chapter 5). Thus, in none of the experimental groups was there a statistically significant difference from the "normal group" in the form of increased glucose tolerance. It is true that a tendency to increased tolerance in relation to the preoperative values was found in the biliary fistula animals in group I A, but the figures were still entirely within the normal range (*cf.* Table 7).

It is therefore evident that an increase in the size of the islet tissue, such as was found in groups I A, II A and IV F a, does not result in distinct signs of increased glucose tolerance. It may be mentioned as an example that not even the animal in which an enormous increase in the islet tissue was found (R. 225, group IV F a) had a tolerance curve differing significantly from the normal (see Table I: 12).

The functional state of the beta cells could not be evaluated with the methods used in the present investigation. Consequently, it is uncertain whether, in the animals with increased islet volume, these cells were in a state of increased insulin output or of increased insulin storage. However this may be, the results of the glucose tolerance tests seem to lend support to SOSKIN & LEVINE's view, *i.e.*, that the glucose administered in this test does not stimulate the pancreas to an increased insulin output.

TABLE 26

Comparison between glucose tolerance areas in the different animal groups

Group	Time[1] (days)	n	Tolerance area mg-min x̄	s	e(x̄)	Test of deviation from normal group t	P ∼
Normal	—	55	4,784	1,525	206	—	—
I A before operation	—	8	5,254	1,498	530	0.816	0.4
I A after operation	26.1	7	4,031	3,143	1,188	1.069	0.3
IV B before operation of I A	—	8	4,728	1,977	699	0.093	0.9
IV B after operation of I A	27.7	9	5,408	1,573	524	1.133	0.3
II A after operation	18.1	7	6,344	2,776	1,049	2.297	0.02*
IV C after operation of II A	21.5	4	3,853	2,250	1,125	1.144	0.3
III A after operation	65.1	8	4,957	1,810	640	0.294	0.8
III C a after operation	100.0[2] 40.6	5	8,584	2,457	1,099	5.063	0.001**
IV D after operation	29.3	4	5,330	213	107	0.710	0.5
IV E after undernutrition	38.4	6	7,015	1,694	692	3.369	0.001**
IV F a before treatment	—	6	5,489	464	190	1.119	0.3
IV F a after treatment	30.7	6	4,713	669	273	0.112	0.9

[1] After operation in groups I, II, III and IV D, length of undernutrition period in group IV E and duration of glucose administration in group IV F a, when the glucose tolerance test was made.

[2] The first figure denotes time after operation, the second figure time after alloxan administration was started.

In those cases in which a decreased islet volume was found in non-diabetic animals, as in group III A, there was also no discernible change in the glucose tolerance curves in relation to the "normal group". It was not until the islet volume had been reduced to a "diabetic" level, as in R. 186, that a pathologic curve was recorded, concurrently with other transient "diabetic" symptoms (*cf.* ALLEN on the effect of subtotal pancreatectomy).

In the three groups in which low glucose tolerance was observed (groups II A, III C a and IV E), the islet volume was increased in the first-mentioned and normal in the other two. As discussed in Chapter 5, undernutrition and liver damage were probably responsible for the decreased tolerance in groups II A and IV E. In group III C a, no such factors were present. In this group there were, however, indications of beta cell damage, despite absence of diabetes and despite normal islet volume

It therefore seems likely that decreased insulin production changed the "setting" of the liver "thermostat" in the animals of the last-mentioned group.

ISLET MORPHOLOGY

The relation between islet size, expressed as m_i in square microns, and islet number, expressed as n_i/BW, in the 15 animal groups studied is shown in Fig. 54. The range in the material is considerable, being 689 to 4,839 μ^2 for m_i and 208 to 1,633 for n_i/BW. It is possible to distinguish three essentially different sets of animals, *i.e.*,

1. Non-diabetic groups with functioning exocrine parenchyma (8 groups: 49 animals)
2. Diabetic groups with functioning exocrine parenchyma (4 groups: 17 animals)
3. Non-diabetic and diabetic groups without functioning exocrine parenchyma (3 groups: 24 animals).

The range of m_i and n_i/BW in these three sets of animals is:

1.	m_i: 2,025—4,839 μ^2	n_i/BW: 361—1,633
2.	m_i: 991—2,424 μ^2	n_i/BW: 208— 896
3.	m_i: 689—2,326 μ^2	n_i/BW: 429—1,453

The correlation between islet size and islet number was calculated from the values in the individual animals, and the corresponding regression lines were constructed. In Fig. 54, the regression line for all 15 groups is denoted as A-A, that for non-diabetic groups with functioning exocrine parenchyma as B-B, that for diabetic groups with functioning exocrine parenchyma as C-C, that for all groups with functioning exocrine parenchyma as D-D, and that for all groups without functioning exocrine parenchyma as E-E. The results of these calculations are also recorded in Table 27.

In the groups with functioning exocrine parenchyma, correlation coefficients were obtained for which the tests of significance showed that the probability was fairly inappreciable of such coefficients occurring by chance in an uncorrelated population. In the groups with atrophic exocrine parenchyma, on the contrary, no such significance was obtained for the correlation coefficients. This lack of correlation between islet number and islet size may be explained by the invasion of the islet tissue by connective tissue, which tended to split the islets into smaller units.

Although I have been unable to find any statements in the literature as

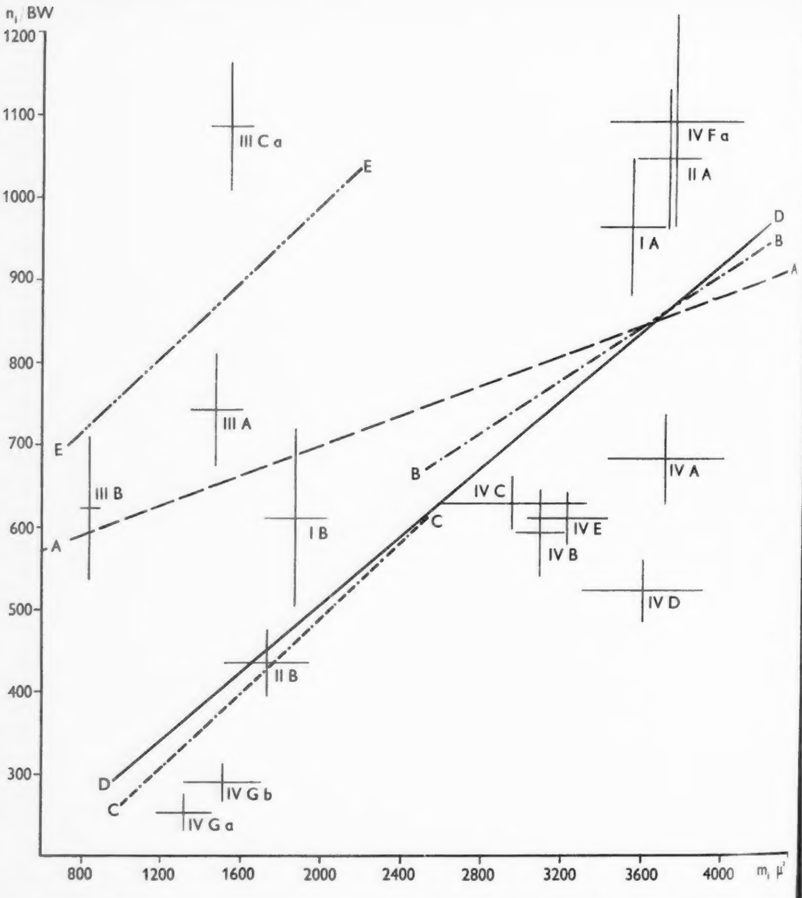

Fig. 54. Correlation between number of counted islets (n_i/BW) and islet size (m_i): all groups. Mean values $\pm e(x)$.
A—A: regression line for all animals. B—B: regression line for non-diabetic animals with functioning exocrine parenchyma. C—C: regression line for diabetic animals. D—D: regression line for diabetic and non-diabetic animals with functioning exocrine parenchyma. E—E: regression line for animals in group III (diabetic and non-diabetic, without functioning exocrine parenchyma).

to calculations of correlation coefficients between islet number and islet size, the aforementioned findings are in agreement with Bensley's general observations in the islet tissue of the guinea-pig and Tejning's in the rat.

The material can also be classified on the basis of the results of the

TABLE 27

Correlations and regression equations in different animal groups for islet size and islet number
$x = m_i\ (\mu^2)$; $y = n_i/BW$ (*cf.* Fig. 54)

Regression line	$\bar{x}$	$\bar{y}$	n	Correlations r	t	P $\sim$	Regression equations
A–A	2,557	749	90	0.32	3.122	0.001***	$y = 518.87 + 0.09\,x$
B–B	3,471	821	49	0.31	2.266	0.02*	$y = 270.85 + 0.16\,x$
C–C	1,609	401	17	0.46	1.980	0.05*	$y = 37.04 + 0.23\,x$
D–D	2,991	713	66	0.62	6.314	0.001***	$y = 91.47 + 0.21\,x$
E–E	1,364	846	24	0.35	1.761	0.1	$y = 532.14 + 0.23\,x$

statistical analyses of the differences between the mean values in the respective animal groups. The groups between which no significant difference exists can then be assembled in larger classes. The following four classes, arranged in rising order of islet size, m_i, are then obtained, and contain the following groups:

		Range	$\bar{x}$
1.	Group III B	689— 938	838 μ^2
2.	Groups I B, II B, III A, III C a, IV G a, IV G b	767—2,424	1,572 μ^2
3.	Groups IV B, IV C	2,025—3,835	3,020 μ^2
4.	Groups I A, II A, IV A, IV D, IV E, IV F a	2,296—4,839	3,583 μ^2

As far as islet size is concerned, the diabetic group III B without functioning exocrine parenchyma thus represents the smallest occurring size. Next in order are the other diabetic groups with functioning exocrine parenchyma and the other groups without functioning exocrine parenchyma. The two largest classes comprise non-diabetic groups with functioning exocrine parenchyma.

Analogously, the following classes are obtained for n_i/BW:

		Range	$\bar{x}$
1.	Groups IV G a, IV G b	208— 334	272
2.	Groups I B, II B, III B, IV B, IV D, IV E	314— 904	565
3.	Groups III A, IV A, IV C	429—1,070	684
4.	Groups I A, II A, III C a, IV F a	602—1,633	1,045

Here, the alloxan-diabetic controls in group IV G comprise the lowest class, the operated alloxan-diabetic groups, the underfed controls and the ureter-ligated animals being the next in order. They are followed by the

untreated controls and the non-diabetic animals in group III A. The highest values for islet number are found in the non-diabetic, bile-flow operated groups I and II, the alloxan-resistant animals in group III C and the glucose-treated animals in group IV F.

The material can also be classified with respect to the order of magnitude of the calculated islet volume, expressed per kg of body weight as V_i/BW:

	Range	x
1. Groups III B, IV G a, IV G b	0.27—0.96	0.53 mm³
2. Groups I B, II B, III A	0.45—2.31	1.21 mm³
3. Groups III C a, IV B, IV C, IV D, IV E	1.25—3.22	2.19 mm³
4. Group IV A	2.85—3.11	2.98 mm³
5. Groups I A, II A, IV F a	2.33—9.48	4.60 mm³

Thus, the alloxan-diabetic controls and the diabetic animals with secondary ligation of the pancreatic duct form the lowest class. Next in order come the diabetic bile-flow operated animals and the non-diabetic animals with the pancreatic duct ligated. The alloxan-resistant animals in group III, the underfed controls to groups I and II and the ureter-ligated group form a "normal" intermediate class. Finally, the bile-flow operated animals in groups I A and II A and the glucose-treated animals in group IV F represent the highest islet volume values (*cf.* Figs. 34, 38 and 52).

It is also possible to distinguish different classes with respect to the alpha cell incidence:

	Range	x
1. Group I A	6.34—22.65	13.51 %
2. Groups II A, III A, IV A, IV B, IV C, IV D, IV E, IV F a	4.63—31.09	18.53 %
3. Group III C a	16.25—51.83	33.77 %
4. Groups I B, II B, III B	24.35—81.28	49.62 %
5. Groups IV G a, IV G b	52.38—89.36	69.20 %

The first class comprises the non-diabetic biliary fistula animals, in which a tendency to a subnormal incidence was present. The second contains the non-diabetic animals with a largely normal incidence. The third class consists of the alloxan-resistant animals in group III, in which there was a somewhat raised alpha cell incidence as a result of supposed beta cell damage (p. 198). The fourth class consists of operated diabetic animals, and the fifth of non-operated diabetic controls.

The lower alpha cell incidence in class 4 than in class 5 has been

ascribed, in the case of groups I B and II B, to a regeneration of beta cells (or non-granular islet cells) produced by the bile flow interventions (*cf.* p. 165). The cause of the lower incidence in group III B in relation to group IV G cannot be determined with certainty. Two conceivable explanations exist. One is that the primary alloxan damage was not as severe in the former animals as in the latter, this supposition being borne out by the comparison between the diabetic condition in the respective groups (*cf.* Tables 15 and 21). The other is that a decrease in the alpha cell incidence took place in connexion with the regenerative processes in the islet tissue associated with ligation of the pancreatic duct.

The following suggestions can be made in a discussion of the *mechanisms* underlying the changes observed in the size of the islet tissue. In the non-diabetic animals in groups I and II, interruption of the normal bile flow resulted in a stimulation to growth of the exocrine pancreas parenchyma. It seems likely that this effect is the primary one, and that enlargement of the islet tissue is secondary. In this exocrine growth, new formation of exocrine tissue probably takes place both from the acini and from the duct epithelium. As BENSLEY has shown in the guinea-pig, this epithelium is present in the whole organ as a finely branched network of extremely thin channels, with which the islets remain in close connexion. According to the same author, this epithelium is "a tissue of a low order of differentiation, which is capable, under proper conditions, of producing by differentiation, and by mitotic division, islets, acini and mucous glands". Consequently, a stimulus to acinar growth may also bring about an increase in the islet tissue.

The possibility of *direct stimulation* of the islets as a result of these bile flow interventions must naturally be left open, although the nature of the underlying mechanisms remains obscure. In this connexion, mention can be made of one factor that has been studied in several investigations by LABARRE (cited by BABKIN). This is the so-called incretin, a component of secretin which, according to LABARRE, has the ability to stimulate insulin secretion and to keep pancreatectomized dogs alive. The criticism of these investigations, which implies that LABARRE had actually included insulin in his secretin preparations, can possibly be refuted by recalling that incretin is stated to be active even on oral administration.

The islet growth in bile flow experiments in diabetic animals may have been produced by the same mechanism as in the non-diabetic animals. As discussed in Chapter 6, new formation of islet tissue from the duct cells is nevertheless uncertain, in view of the alloxan damage to this epithelium demonstrated by some workers (*cf.* p. 164).

It is true that, after ligation of the pancreatic duct, atrophy of the islet tissue unquestionably takes place, owing to degeneration in connexion with the marked fibrosis of the organ. It is nevertheless also evident, from this and previous investigations, that signs are present of concurrent regenerative processes. Here as well, the duct epithelium plays an important role as the mother substance for the newly formed islets.

It is possible that, in animals with less tendency to fibrosis than the rabbit, these regenerative processes cause the hyperplasia of the islet tissue described by some authors (see Chapter 1).

Thus, the results of both the bile flow experiments and ligation of the pancreatic duct suggest that the *ambipotency* existing in the duct epithelium during foetal development can, under special conditions, also appear postnatally.

ISLET HAEMORRHAGE

Islet haemorrhage was observed in four animals in my material; two belonged to group II A (R. 213 and 214), one to group I B (R. 194) and one to group IV F a (R. 229). The extent was variable, the haemorrhages being numerous in the two first-mentioned cases but less frequent in the two last-mentioned. The appearance of such islets is illustrated in Figs. 29, 30 and 31.

Islet haemorrhage is a rare phenomenon, and data in the literature regarding it are sparse. In LANDO's thoroughly investigated 23 cases of pancreatic lesions in cirrhosis of the liver, it is stated that islet haemorrhages "waren sehr häufig", in addition to other lesions (fibrosis) particularly in the exocrine parenchyma. In a similar study of the pancreas in liver cirrhosis made by POGGENPOHL, a few haemorrhagic islets were present in 4 out of 22 cases. KRAUS stated that islet haemorrhage may occur in diabetes in man, and that they are possibly associated with arteriosclerotic changes. WARREN & LECOMPTE have, however, expressed the view that when islet haemorrhage has been described, it has probably been either a post mortem change or an artefact. BARRON & STATE, in a study of the effect of prolonged glucose administration in 6 dogs, found numerous diffuse haemorrhages and haemorrhagic necroses in the islets of one animal.

In embryonic pancreatic tissue, LAGUESSE observed islet haemorrhage in the "primary" islet primordia described by him. This observation has been confirmed by VAN CAMPENHOUT and by SIWE (*cf.* BARGMANN). LAGUESSE stated the haemorrhages to be a destruction phenomenon

According to van Campenhout, they are caused by rupture of vessels due to rapid growth of the islet primordia. This view is shared by Siwe.

The origin of the islet haemorrhages observed in my material cannot be established with certainty. In the three cases in groups II A and I B, a haemorrhagic diathesis resulting from hypoprothrombinaemia associated with interruption of the bile flow might have been responsible. These animals did not, however, exhibit any signs of haemorrhage in other tissues. Another conceivable cause in these three cases is the presence of liver cirrhosis, in accordance with the observations of Lando and of Poggenpohl.

There is, however, another factor that is common to all four cases, *i.e.*, the increase in size of the islet tissue. In agreement with the opinion of van Campenhout, a likely explanation is that the islet haemorrhages were an expression of proceeding islet growth. It is true that the phenomenon was observed in only four animals, which may have been especially vulnerable. Nevertheless, against the background of its great rarity, this observation lends additional support to the view that, under the experimental conditions in question, the islet tissue has been stimulated to growth.

GENERAL SUMMARY AND CONCLUSIONS

On the basis of earlier investigations suggesting an increase in the islet tissue of the pancreas in experimental biliary fistula in dogs, the object of the present investigation was the following. To analyze, by means of quantitative methods, the effect on glucose tolerance and pancreas morphology, in non-diabetic and alloxan-diabetic rabbits, of:

1. *Biliary fistula*
2. *Ligation of the common bile duct.*

The results of these experiments showed an increase in size of the whole pancreas, as well as of the islet tissue. This motivated analysis of the glucose tolerance and pancreas morphology with the same methods, in non-diabetic and alloxan-diabetic rabbits, after:

3. *Ligation of the pancreatic duct,* as well as
4. *The effect of alloxan after ligation of the pancreatic duct.*

The results of these experiments were compared with those obtained after:

5. *Long-term parenteral administration of glucose.*

CHAPTER 1. A brief survey of some earlier investigations is given under the following headings:

1. *The liver and diabetes:* liver function in diabetes, diabetic signs in liver disease, and effect on diabetes of secondary hepatic lesions (Summary: p. 22).

2. *The islet tissue in pancreatic fibrosis and pancreatic disease:* different opinions as to size of the islet tissue after ligation of the pancreatic duct, rarity of islet lesions in pancreatic disease (Summary: p. 26).

3. *Effect of alloxan after ligation of pancreatic duct:* resistance to diabetogenic effect of alloxan in animals with fibrosis of the exocrine pancreas parenchyma, different hypotheses regarding the cause of this resistance (Summary: p. 29).

4. *Factors affecting the size of the islet tissue:* increase in size of islet tissue following high-carbohydrate diets, administration of glucose and of anterior pituitary hormones (Summary: p. 32).

CHAPTER 2. The experimental *material* consisted of 144 rabbits, divided into three experimental groups and one control group (Table 1). In 92 cases, a more or less complete micromorphologic examination of the pancreas was made; in the remaining 52 animals no such examination was performed for various reasons.

CHAPTER 3. The *methods* used are described. An intravenous *glucose tolerance test* was used, and the results evaluated by calculating the glucose tolerance area with a rapid and simple technique (Table 2, Fig. 1). A modification of ROUS & MCMASTER's method was applied for creation of a *biliary fistula*. A *replacement fluid* of approximately the same electrolyte composition as the bile was administered parenterally.

The whole pancreas was fixed in Bouin's fluid and stained according to Gomori. *Quantitative micromorphologic analyses* of the total pancreas parenchyma, as well as of the islet tissue, were made on sections of the serial-sectioned organ at intervals of 1,200 μ. This amounted to about 12 sections per animal. The *islet size* was calculated from measurement of the cut surface of a sample comprising about 200 islets from each animal. The *total number of islets* in each section was determined by systematic counting. The incidence of *alpha cells* was determined by a differential count of at least 1,000 islet cells from each animal.

Pieces of tissue from the liver, kidneys, duodenum and adrenal glands were examined for their content of *glycogen, lipids* and *alkaline phosphatase*. A semi-quantitative approximate grading was used.

CHAPTER 4. The methods are discussed. *Previous methods* used in evaluation of glucose tolerance tests, in creation of a biliary fistula and in quantitative micromorphologic analyses are described, and compared with the *present methods*. The difficulties in determining the absolute number of islets in the pancreas are analyzed. An approximative application of FLODERUS' reduction formula is described (Figs. 14 and 15).

CHAPTER 5. *Bile flow experiments* were performed on 41 *non-diabetic* animals. A *biliary fistula* was created in 19 cases, and *ligation of the common bile duct* was performed in 22. Quantitative micromorphologic analyses were made in 20 of these cases, and the results compared with those in 17 similarly fed controls.

In the biliary fistula animals, a slightly increased *glucose tolerance* in comparison to the controls was recorded postoperatively. No such increase occurred in the biliary stasis group. Decreased tolerance was re-

corded in some animals, and was interpreted as a result of impaired glycogenesis and glycogenolysis due to liver damage.

A reduction in the *adipose tissue* of a varying degree of severity was observed in both groups of operated animals. It is considered as improbable that decreased absorption of the quantitatively inappreciable dietary fat is responsible for this feature. A disturbance in lipogenesis, as a result of liver cell damage, is suggested as the cause.

An increase in the *parenchymal volume of the whole pancreas* was observed in both the biliary fistula and the biliary stasis animals (Fig. 32). It amounted to about 60 per cent in comparison to the controls. This phenomenon does not seem to have been observed in earlier investigations. A hypothetical explanation is given on the basis of some previous observations showing an inhibitory effect of bile on pancreatic secretion.

An increase in the *size of the islet tissue* was present in both groups. It amounted to about 100 per cent in relation to the controls (Fig. 34). The increase could be ascribed more to an increase in the number of the islets than to an increase in size of the individual islets.

The *alpha cell incidence* was within the normal range of variation in both groups, although it was somewhat lower in the biliary fistula animals than in the corresponding controls.

Islet haemorrhage was observed in two animals with biliary stasis (Figs. 29—31).

CHAPTER 6. *Bile flow experiments* were performed on 11 *alloxan-diabetic* animals. Quantitative micromorphologic analyses were made in 8 cases, and the results compared with those in 9 non-operated, alloxan-diabetic controls.

The *diabetic condition*, evaluated by the urinary excretion of glucose and the insulin requirement, showed an improvement in all operated animals. The cause is considered to be the reduced caloric intake postoperatively, and possibly an increase in endogenous insulin production.

The *size of the islet tissue* was considerably less in the alloxan-diabetic controls than in non-diabetic animals. In the operated alloxan-diabetic animals, an increase in islet volume was present; it amounted to 150 per cent in relation to the non-operated, diabetic controls (Fig. 38).

The *alpha cell incidence* showed a marked tendency to normalization in the operated animals. The pancreas did not exhibit the typical features of alloxan damage seen in the controls (Figs. 39—40). In some the islet tissue had a practically normal appearance; in others fairly numerous

"*Mantelinseln*" were present (Figs. 41—44). *Islet haemorrhage* was observed in one animal.

The problem of *regeneration of islet tissue* in alloxan diabetes is discussed against the background of earlier investigations. Such regeneration has not previously been observed with certainty, except in the guinea-pig. New formation of islet cells from beta cells undamaged by alloxan is regarded as the most probable explanation of the signs of regeneration seen in the present material. This is because the damage to the duct epithelium described by some workers may make regeneration from this epithelium impossible.

No change in the diabetogenic effect of alloxan was observed in two animals with biliary obstruction (Table 22).

CHAPTER 7. *Ligation of the pancreatic duct* was performed in 34 animals. Quantitative micromorphologic analyses were made in 24 cases. The effect of the intervention was studied in 10 *non-diabetic* and in 5 *alloxan-diabetic* animals. The effect of *alloxan after ligation of the duct* was studied in 15 animals; alloxan was given about 68 days after operation in 9 cases, and after 11 days in 6.

With one exception, no postoperative nutritional disturbance was observed. The *diastasuria* following the intervention disappeared after 4 to 6 days (Table 19).

The *glucose tolerance*, studied in 9 non-diabetic animals, was normal except in one case, in which an abnormal "diabetic" curve was found.

The *diabetic condition* of the alloxan-diabetic animals was uninfluenced by ligation of the pancreatic duct.

Complete *resistance to the diabetogenic effect of alloxan* was observed in all animals to which it was given late after operation, and partial resistance when the substance was given soon after operation (Table 22). In the former animals, glucose tolerance tests showed abnormal curves with a high level and slow fall, but normal fasting values.

At autopsy, complete *atrophy of the exocrine parenchyma* of the pancreas was found in all long-term experiments, except in one animal, in which an inappreciable remains of the gland was present in the vicinity of the pylorus (Figs. 46—48, 53).

Analysis of the *islet tissue* showed a decrease in islet volume in the non-diabetic animals, amounting to 40 to 55 per cent in relation to the controls. In the diabetic animals, the size of the islet tissue was the same as that in alloxan-diabetic controls. In the completely alloxan-resistant group, the

islet tissue was larger than that of both the non-diabetic and diabetic groups with the pancreatic duct ligated (Fig. 52).

The *alpha cell incidence* was normal in the non-diabetic group, high in the diabetic group and intermediate in the alloxan-resistant group.

In no case were signs of liver damage observed. The *glycogen* and *lipid content* of the liver cells was the same as in the controls (Table 12).

The results are interpreted as an indication that, after ligation of the pancreatic duct, both regressive and progressive changes occur in the islet tissue. At the stage at which the present animals were studied post mortem, no signs of hyperplasia of the islet tissue were visible.

The *resistance to alloxan after pancreatic duct ligation* is discussed. It is considered that it cannot be explained by islet hyperplasia, impaired blood supply or liver damage, as postulated by earlier workers. It is suggested that the *foetal nature* of the islet tissue after ligation of the duct may be the factor responsible. This hypothesis is supported by several previous investigations. Absence of active secretion from the acinar parenchyma is regarded as a possible additional factor of importance.

CHAPTER 8. The effect of *glucose administration* was studied in 12 animals. Quantitative micromorphologic analyses of the *islet tissue* in 6 of them showed a considerable increase in islet volume in some animals, and a slight increase or none in others. The mean value of the islet volume was similar to that obtained in the bile flow experiments, although the scattering was much greater (Fig. 34). *Islet haemorrhage* was observed in one animal.

The *glucose tolerance test* made after 4 weeks' glucose administration showed a normal curve in every case.

The *diabetogenic effect of alloxan* in 6 animals after 4 weeks' glucose administration was found to be the same as in previously untreated animals (Table 22).

CHAPTER 9. The results of the *glucose tolerance tests* are discussed. In the present material, no correlation was found between the type of glucose tolerance curve and increased size of the islet tissue. This is considered to support the view of SOSKIN and co-workers that the glucose administered does not stimulate the pancreas to an additional secretion of insulin. In the biliary fistula group with a large islet volume, a tendency was noted to increased tolerance, although the values lay within the normal range. Low glucose tolerance was observed in undernutrition and in the presence of liver cell damage. This also applied when there was a subtotal decrease in islet volume, and when signs of beta cell damage without diabetes were present.

An account is given of the relation between *islet size and islet number* (Table 27, Fig. 54). The material is classified according to the size of islet tissue and according to the alpha cell incidence.

The *mechanisms* underlying the changes observed in islet tissue are discussed. It is suggested that, under certain conditions, regeneration from the duct epithelium may take place postnatally. Factors directly stimulating the islet cells are also regarded as conceivable.

The phenomenon of *islet haemorrhage* is discussed. Its extreme rarity in previous investigations is recalled. In agreement with the views of some earlier workers, it is considered to be an expression of proceeding islet growth.

CONCLUSIONS

On the basis of the results of the present investigations, the following conclusions are drawn:

1. In non-diabetic rabbits, the absence of bile in the intestine, as is the case in biliary fistula or in ligation of the common bile duct, leads to an increase in the total pancreas parenchyma and in the size of the islet tissue.

2. In alloxan-diabetic rabbits, the absence of bile in the intestine under the same conditions results in an improvement in the diabetic condition, an increase in size of the islet tissue, signs of regeneration of islet cells, appearance of "Mantelinseln" and a trend to normalization of the alpha cell incidence.

3. In non-diabetic rabbits, ligation of the pancreatic duct produces, when analyses are made two to three months later, a considerable decrease in size of the islet tissue, although the histologic features indicate the presence of both regressive and progressive changes.

4. In alloxan-diabetic rabbits, ligation of the pancreatic duct results in no change in the diabetic condition, nor in any change in size of the islet tissue in comparison with alloxan-diabetic controls.

5. Ligation of the pancreatic duct in the rabbit leads to complete resistance to the diabetogenic effect of alloxan, when administered about two months later. The resistance is probably due to the foetal nature of the islet tissue after this intervention.

6. Glucose administration subcutaneously for four weeks leads to an average increase in size of the islet tissue in the rabbit, although the individual variation is considerable.

7. Rabbits submitted to treatment (bile flow obstruction, glucose administration) found to lead to islet hyperplasia are not resistant to the diabetogenic effect of alloxan.

8. The glucose tolerance (intravenous test) is not significantly increased in rabbits with islet hyperplasia, although a tendency to increased tolerance is found in biliary fistula animals. Low glucose tolerance is present in undernutrition, in liver damage, in subtotal reduction of the islet tissue, and in animals with signs of beta cell damage without reduction in size of the islet tissue and without diabetes.

9. New formation of islet tissue from the duct epithelium in the rabbit is possible even postnatally under certain conditions. Regeneration may also occur by direct stimulation of islet cells. Islet haemorrhage may be a sign of increased islet growth.

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APPENDIX

TABLES I: 1—I: 12

Intravenous glucose tolerance tests. Blood sugar, mg/100 ml

TABLE I: 1

Group I A before operation (*cf.* Fig. 19)

Animal no.	Time (min) 0	5	10	15	30	45	60	75	90	Tolerance area (mg-min)
R. 101	106	218	199	185	174	150	124	—	104	4,485
R. 103	96	196	168	163	146	131	117	—	108	3,168
R. 106	103	195	195	188	165	127	122	—	110	3,960
R. 108	121	235	207	203	172	142	121	—	110	4,858
R. 110	107	214	203	199	178	149	131	110	103	4,710
R. 129	123	230	215	205	191	175	157	145	131	6,990
R. 134	118	262	235	225	184	173	152	138	122	6,950
R. 136	111	247	231	215	192	170	154	143	125	6,910
$\bar{x}$	111	225	207	198	175	152	135	134	114	5,254

TABLE I: 2

Group IV B before operation of paired animals in group I A (*cf.* Fig. 20)

Animal no.	Time (min) 0	5	10	15	30	45	60	75	90	Tolerance area (mg-min)
R. 102	106	212	195	173	153	134	125	—	110	3,918
R. 104	96	177	170	155	127	106	101	—	96	1,803
R. 107	74	186	190	181	148	134	117	—	88	3,303
R. 109	99	203	199	174	148	121	113	—	88	3,078
R. 111	103	216	203	197	174	163	149	133	112	5,560
R. 133	114	232	210	210	173	170	147	135	117	6,080
R. 135	108	250	240	226	191	177	159	143	133	7,335
R. 137	93	250	230	210	196	174	160	135	118	6,745
$\bar{x}$	99	216	205	191	164	147	134	137	108	4,728

TABLE I: 3

Group I A after operation (*cf.* Fig. 21)

Animal no.	Time (min) 0	5	10	15	30	45	60	75	90	Tolerance area (mg-min)
R. 101	103	177	161	156	131	120	110	106	99	2,285
R. 103	100	201	174	181	160	130	97	113	107	3,335
R. 106	104	199	181	176	143	116	108	105	94	2,780
R. 110	94	181	176	135	141	125	117	103	98	2,425
R. 122	96	169	143	132	112	102	97	93	95	1,090
R. 129	133	—	253	217	221	213	202	205	193	10,275
R. 134	96	218	211	207	188	163	149	137	117	6,025
$\bar{x}$	104	191	186	172	157	138	126	123	115	4,031

TABLE I: 4

Group IV B after operation of paired animals in group I A (*cf.* Fig. 22)

Animal no.	Time (min) 0	5	10	15	30	45	60	75	90	Tolerance area (mg·min)
R. 102	103	222	233	204	192	174	148	141	134	6,680
R. 104	100	228	200	191	151	136	113	102	96	3,580
R. 107	105	220	205	197	181	153	129	123	108	5,055
R. 109	99	246	214	209	180	152	126	124	117	5,375
R. 111	126	242	224	215	191	167	152	141	127	6,910
R. 123	94	189	175	167	129	122	112	93	91	2,435
R. 133	96	214	207	198	174	149	127	110	98	5,485
R. 135	104	229	215	207	194	184	163	150	136	7,235
R. 137	96	234	233	218	187	159	141	127	113	5,920
x̄	103	225	212	201	175	155	135	123	113	5,408

TABLE I: 5

Group II A after operation (*cf.* Fig. 26)

Animal no.	Time (min) 0	5	10	15	30	45	60	75	90	Tolerance area (mg·min)
R. 113	108	194	189	179	170	156	149	131	119	5,160
R. 114	92	188	178	167	145	124	112	106	104	2,865
R. 116	126	232	228	222	219	200	213	201	194	10,685
R. 156	105	203	181	177	152	145	134	120	119	4,290
R. 160	103	237	203	185	167	149	137	126	116	5,005
R. 166	117	218	200	197	187	184	163	158	160	7,510
R. 233	113	241	220	210	200	191	181	178	174	8,895
x̄	109	216	200	191	177	164	156	146	141	6,344

TABLE I: 6

Group IV C after operation of control animals in group II A

Animal no.	Time (min) 0	5	10	15	30	45	60	75	90	Tolerance area (mg·min)
R. 115	87	188	169	167	143	122	111	95	91	2,595
R. 127	113	256	247	218	194	176	150	131	118	6,860
R. 157	108	229	205	195	—	153	139	124	118	4,210
R. 161	93	203	184	145	124	100	92	85	81	1,745
x̄	100	219	201	181	154	138	123	109	102	3,853

TABLE I: 7

Group IV E after the undernutrition period (*cf.* Fig. 27)

Animal no.	Time (min) 0	5	10	15	30	45	60	75	90	Tolerance area (mg-min)
R. 113	113	230	216	218	190	182	157	132	121	6,770
R. 116	114	242	216	208	180	152	149	134	128	6,155
R. 169	113	251	246	240	223	207	194	171	157	9,752
R. 170	113	251	241	229	203	187	171	154	143	8,250
R. 171	89	227	214	202	185	162	143	139	127	6,038
R. 172	97	228	217	201	178	154	136	116	107	5,123
$\bar{x}$	107	238	225	216	193	174	158	141	131	7,015

TABLE I: 8

Group III A after operation

Animal no.	Time (min) 0	5	10	15	30	45	60	75	90	Tolerance area (mg-min)
R. 177	97	222	202	190	162	149	110	109	107	4,075
R. 182	94	224	204	188	159	138	123	103	94	3,895
R. 183	89	192	176	167	123	103	88	82	85	1,900
R. 205	95	218	188	183	173	156	131	116	106	4,590
R. 206	110	237	232	214	203	181	161	133	126	7,145
R. 209	116	211	208	201	185	161	154	139	129	6,285
R. 210	107	212	199	189	171	148	131	122	110	4,745
R. 185	109	245	225	211	195	179	160	140	124	7,020
(R. 186[1]	208	301	302	295	276	259	251	244	226	16,385)
$\bar{x}$	102	220	204	193	171	152	132	118	110	4,957

[1] Not included in the calculation of $\bar{x}$

TABLE I: 9

Group III C a after operation and alloxan administration (*cf.* Fig. 51)

Animal no.	Time (min) 0	5	10	15	30	45	60	75	90	Tolerance area (mg-min)
R. 195	99	201	—	—	188	—	156	—	136	6,425
R. 196	100	219	—	—	210	—	199	—	181	9,603
R. 197	103	220	—	—	188	—	161	—	139	6,958
R. 198	103	236	—	—	185	—	170	—	145	7,521
R. 199	109	260	—	—	241	—	219	—	210	12,413
$\bar{x}$	103	227			202		181		162	8,584

TABLE I: 10

Group IV D after operation

Animal no.	Time (min) 0	5	10	15	30	45	60	75	90	Tolerance area (mg-min)
R. 153	103	227	214	189	175	154	141	126	112	5,245
R. 154	99	233	225	207	182	156	143	127	106	5,570
R. 139	117	264	245	225	161	141	124	125	113	5,425
R. 163	116	228	210	197	176	149	129	118	112	5,080
$\bar{x}$	109	238	224	205	174	150	134	124	111	5,330

TABLE I: 11

Group IV F a before glucose administration

Animal no.	Time (min) 0	5	10	15	30	45	60	75	90	Tolerance area (mg-min)
R. 225	117	250	227	196	173	143	129	124	120	5,350
R. 226	112	211	190	191	173	156	142	132	121	5,395
R. 227	109	225	203	205	182	164	149	131	126	6,060
R. 228	102	197	187	182	162	159	143	125	113	4,790
R. 229	98	212	199	195	177	154	140	130	124	5,380
R. 230	106	240	214	206	177	158	141	136	126	5,960
$\bar{x}$	107	223	203	196	174	156	141	130	122	5,489

TABLE I: 12

Group IV F b after glucose administration

Animal no.	Time (min) 0	5	10	15	30	45	60	75	90	Tolerance area (mg-min)
R. 225	120	246	222	210	166	141	129	121	119	5,280
R. 226	109	216	198	189	158	143	127	119	122	4,585
R. 227	100	222	191	183	151	131	118	112	101	3,590
R. 228	98	225	205	192	165	149	137	121	113	4,845
R. 229	103	229	212	200	181	156	140	121	118	5,475
R. 230	98	228	218	198	165	138	121	120	109	4,505
$\bar{x}$	105	228	208	195	164	143	129	119	114	4,713

TABLES II: 1—II: 15

Body size and pancreas size of individual animals

TABLE II: 1

Group I A

Animal no.	BW kg	BS dm²	PW g	PW/BW g		P mm²	p mm²	V_p mm³	V_p/BW mm³
R. 101	2.14	16.0	4.69	2.19	D	163,635	1,136.3	1,363.6	637.2
					L	82,021	569.6	683.5	319.4
					DL	245,656	1,705.9	2,047.1	956.6
R. 103	2.49	17.1	4.90	1.97	D	132,110	917.4	1,100.9	442.1
					L	93,086	646.4	775.7	311.5
					DL	225,196	1,563.9	1,876.7	753.7
R. 106	2.53	17.2	3.43	1.36	D	94,547	656.6	787.9	311.4
					L	71,176	494.3	593.2	234.5
					DL	165,723	1,150.9	1,381.1	545.9
R. 108	1.52	13.7	3.14	2.07	D	69,515	482.7	579.2	381.1
					L	54,455	378.2	453.8	298.6
					DL	123,970	860.9	1,033.1	679.7
R. 110	2.15	16.0	5.46	2.54	D	70,775	491.5	589.8	274.3
					L	47,352	328.8	394.6	183.5
					DL	118,127	820.3	984.4	457.9
R. 122	1.45	13.5	3.56	2.46	D	87,765	609.5	731.4	504.4
					L	67,642	469.7	563.6	388.7
					DL	155,407	1,079.2	1,295.0	893.1
R. 129	1.74	14.6	3.79	2.18	D	74,722	518.9	622.7	357.9
					L	31,801	220.8	265.0	152.3
					DL	106,523	739.7	887.6	510.1
R. 134	2.72	17.7	7.44	2.73	D	87,862	610.2	732.2	269.2
					L	68,050	472.6	567.1	208.5
					DL	155,912	1,082.7	1,299.2	477.6
R. 136	2.27	16.4	6.48	2.85	D	94,391	655.5	786.6	346.5
					L	115,374	801.2	961.4	423.5
					DL	209,765	1,456.7	1,748.0	770.0

TABLE II: 2

Group IV B

Animal no.	BW kg	BS dm²	PW g	PW/BW g		P mm²	p mm²	V_p mm³	V_p/BW mm³
R. 102	2.30	16.5	3.71	1.61	D	70,394	488.8	586.6	255.0
					L	65,415	454.3	545.2	237.0
					DL	135,809	943.1	1,131.7	492.0
R. 104	2.28	16.4	2.81	1.23	D	59,113	410.5	492.6	216.1
					L	45,372	315.1	378.1	165.8
					DL	104,485	725.6	870.7	381.9
R. 107	2.88	18.1	3.83	1.33	D	88,703	616.0	739.2	256.7
					L	65,516	455.0	546.0	189.6
					DL	154,219	1,071.0	1,285.2	446.3
R. 109	2.63	17.4	3.42	1.30	D	83,066	576.8	692.2	263.2
					L	65,903	457.7	549.2	208.8
					DL	148,969	1,034.5	1,241.4	472.0
R. 111	2.54	17.2	2.94	1.16	D	56,040	389.2	467.0	183.9
					L	47,846	332.3	398.8	157.0
					DL	103,886	721.4	865.7	340.8
R. 123	1.98	15.4	3.69	1.86	D	82,574	573.4	688.1	347.5
					L	38,059	264.3	317.2	160.2
					DL	120,628	837.7	1,005.2	507.7
R. 133	2.59	17.3	3.80	1.47	D	82,929	575.9	691.1	266.8
					L	36,795	255.5	306.6	118.4
					DL	119,724	831.4	997.7	385.2
R. 135	2.98	18.4	3.36	1.13	D	36,072	250.5	300.6	100.9
					L	32,633	226.6	271.9	91.2
					DL	68,705	477.1	572.5	192.1
R. 137	2.38	16.7	4.83	2.03	D	76,459	531.0	637.2	267.7
					L	24,373	169.3	203.2	85.4
					DL	100,832	700.2	840.2	353.0

TABLE II: 3

Group II A

Animal no.	BW kg	BS dm^2	PW g	PW/BW g		P mm^2	p mm^2	V_p mm^3	V_p/BW mm^3
R. 113	2.51	17.1	6.23	2.48	D	172,947	1,201.0	1,441.2	574.2
					L	45,084	313.1	375.7	149.7
					DL	218,031	1,514.1	1,816.9	723.9
R. 114	1.63	14.2	5.81	3.56	D	113,942	791.3	949.6	582.6
					L	70,575	490.1	588.1	360.8
					DL	184,517	1,281.4	1,537.7	943.4
R. 116	2.41	16.8	10.86	4.50	D	135,022	937.7	1,125.2	466.9
					L	103,067	715.7	858.8	356.3
					DL	238,089	1,653.4	1,984.1	823.3
R. 125	2.80	17.9	6.73	2.40	D	102,430	711.3	853.6	304.9
					L	71,720	498.1	597.7	213.5
					DL	174,150	1,209.4	1,451.3	518.3
R. 126	2.84	18.0	6.76	2.38	D	158,852	1,103.1	1,323.7	466.1
					L	66,711	463.3	556.0	195.8
					DL	225,563	1,566.4	1,879.7	661.9
R. 156	2.48	17.0	5.78	2.33	D	85,545	594.1	712.9	287.5
					L	44,865	311.6	373.9	150.8
					DL	130,410	905.6	1,086.7	438.2
R. 160	2.45	16.9	9.09	3.71	D	78,931	548.1	657.7	268.4
					L	41,953	291.3	349.6	142.7
					DL	120,884	839.5	1,007.4	411.2
R. 166	2.42	16.8	7.60	3.14	D	99,049	687.8	825.4	341.1
					L	74,546	517.7	621.2	256.7
					DL	173,595	1,205.5	1,446.6	597.8
R. 213	2.35	16.6	5.65	2.40	D	149,303	1,036.8	1,244.2	529.4
					L	67,317	467.5	561.0	238.7
					DL	216,620	1,504.3	1,805.2	768.2
R. 214	2.49	17.1	6.07	2.44	D	169,151	1,174.7	1,409.6	566.1
					L	83,524	580.0	696.0	279.5
					DL	252,675	1,754.7	2,105.6	845.6
R. 219	1.82	14.8	5.19	2.85	D	107,534	746.8	896.2	492.4
					L	70,161	487.2	584.6	321.2
					DL	177,695	1,234.0	1,480.8	813.6

TABLE II: 4

Group IV C

Animal no.	BW kg	BS dm²	PW g	PW/BW g		P mm²	p mm²	V_p mm³	V_p/BW mm³
R. 157	2.91	18.2	4.67	1.60	D	110,805	769.5	923.4	317.3
					L	22,568	156.7	188.0	64.6
					DL	133,373	926.2	1,111.4	381.9
R. 161	2.85	18.0	3.58	1.26	D	67,446	468.4	562.1	197.2
					L	69,512	482.7	579.2	203.2
					DL	136,958	951.1	1,141.3	400.5
R. 115	2.24	16.3	4.02	1.79	D	85,523	593.9	712.7	318.2
					L	49,811	345.9	415.1	185.3
					DL	135,334	939.8	1,127.8	503.5
R. 127	3.36	19.4	5.60	1.67	D	94,012	652.9	783.5	233.2
					L	30,930	214.8	257.8	76.7
					DL	124,942	867.7	1,041.2	309.9

TABLE II: 5

Group IV E

Animal no.	BW kg	BS dm²	PW g	PW/BW g		P mm²	p mm²	V_p mm³	V_p/BW mm³
R. 169	2.20	16.1	3.04	1.38	D	79,046	548.9	658.7	299.4
					L	61,990	430.4	516.5	234.8
					DL	141,036	979.4	1,175.3	534.2
R. 170	2.36	16.6	3.68	1.56	D	100,316	696.6	835.9	354.2
					L	51,650	358.7	430.4	182.4
					DL	151,966	1,055.3	1,266.4	536.6
R. 171	2.34	16.6	2.90	1.24	D	88,860	617.1	740.5	316.5
					L	35,014	243.2	291.8	124.7
					DL	123,874	860.2	1,032.2	441.1
R. 172	2.28	16.4	2.58	1.13	D	77,766	540.0	648.0	284.2
					L	44,485	308.9	370.7	162.6
					DL	122,251	849.0	1,018.8	446.8

TABLE II: 6

Group IV D

Animal no.	BW kg	BS dm^2	PW g	PW/BW g		P mm^2	p mm^2	V_p mm^3	V_p/BW mm^3
R. 152	2.85	18.0	3.89	1.36	D	82,675	574.1	688.9	241.7
					L	45,330	314.8	377.8	132.6
					DL	128,005	888.9	1,066.7	374.3
R. 153	2.61	17.4	3.96	1.52	D	82,628	573.8	688.6	263.8
					L	44,545	309.3	371.2	142.2
					DL	127,173	883.1	1,059.7	406.0
R. 154	3.53	19.8	6.77	1.92	D	94,366	655.3	786.4	222.8
					L	46,611	323.7	388.4	110.0
					DL	140,977	979.0	1,174.8	332.8
R. 155	3.26	19.2	5.57	1.71	D	94,621	657.1	788.5	241.9
					L	60,630	421.0	505.2	155.0
					DL	155,251	1,078.1	1,293.7	396.8

TABLE II: 7

Group IV A

Animal no.	BW kg	BS dm^2	PW g	PW/BW g		P mm^2	p mm^2	V_p mm^3	V_p/BW mm^3
R. 178	3.20	19.0	5.29	1.65	D	83,966	583.1	699.7	218.7
					L	67,328	467.6	561.1	175.3
					DL	151,294	1,050.7	1,260.8	394.0
R. 179	3.02	18.5	3.84	1.27	D	85,037	590.5	708.6	234.6
					L	49,570	344.2	413.0	136.8
					DL	134,607	934.8	1,121.8	371.5
R. 241	2.67	17.6	3.79	1.42	D	91,350	634.4	761.3	285.1
					L	66,795	463.9	556.7	208.5
					DL	158,145	1,098.2	1,317.8	493.6
R. 242	3.28	19.2	4.30	1.31	D	88,626	615.5	738.6	225.2
					L	96,735	671.8	806.2	245.8
					DL	185,361	1,287.2	1,544.6	470.9

TABLE II: 8

Group I B

Animal no.	BW kg	BS dm²	PW g	PW/BW g
R. 14	1.40	13.3	—	—
R. 193	2.83	18.0	6.55	2.31
R. 194	2.35	16.6	5.26	2.24
R. 216	2.41	16.8	4.49	1.86
R. 180	3.04	18.6	4.25	1.40

TABLE II: 9

Group II B

Animal no.	BW kg	BS dm²	PW g	PW/BW g
R. 148	3.22	19.1	4.63	1.44
R. 149	3.15	18.9	5.86	1.86
R. 174	2.83	18.0	5.53	1.95

TABLE II: 10

Group IV G a

Animal no.	BW kg	BS dm²	PW g	PW/BW g
R. 143	2.49	17.1	3.69	1.48
R. 146	3.38	19.5	5.21	1.54
R. 147	3.07	18.7	4.50	1.47
R. 165	3.62	20.0	5.21	1.44

TABLE II: 11

Group IV G b

Animal no.	BW kg	BS dm²	PW g	PW/BW g
R. 243	3.52	19.8	3.35	0.95
R. 245	2.64	17.5	2.68	1.02
R. 246	4.00	20.9	3.89	0.97
R. 247	3.88	20.6	5.18	1.34
R. 248	3.33	19.3	3.64	1.09

TABLE II: 12

Group III A

Animal no.	BW kg	BS dm²	PW g	PW/BW g
R. 177	2.62	17.4	2.10	0.80
R. 182	2.70	17.6	2.38	0.88
R. 183	2.99	18.5	2.70	0.90
R. 205	2.82	18.0	5.49	1.95
R. 206	2.61	17.4	6.59	2.52
R. 209	2.44	16.9	4.88	2.00
R. 210	2.67	17.6	5.39	2.02
R. 185	2.51	17.1	4.38	1.75
R. 186	3.51	19.8	4.63	1.32
R. 191	3.94	20.7	6.96	1.77

TABLE II: 13

Group III B

Animal no.	BW kg	BS dm²	PW g	PW/BW g
R. 201	3.16	18.9	4.35	1.38
R. 202	3.04	18.6	4.72	1.55
R. 217	3.15	18.9	7.86	2.50
R. 218	3.11	18.8	5.36	1.72
R. 215	3.40	19.5	4.62	1.36

TABLE II: 14

Group III C a

Animal no.	BW kg	BS dm^2	PW g	PW/BW g
R. 211	2.34	16.6	4.70	2.01
R. 212	2.96	18.4	7.20	2.43
R. 195	2.90	18.2	6.29	2.17
R. 196	2.36	16.6	3.62	1.53
R. 197	3.24	19.1	9.40	2.90
R. 198	3.02	18.5	6.26	2.07
R. 199	3.18	19.0	5.71	1.80
R. 200	2.89	18.2	6.67	2.31
R. 207	2.83	18.0	5.18	1.83

TABLE II: 15

Group IV F a

Animal no.	BW kg	BS dm^2	PW g	PW/BW g
R. 225	2.73	17.7	3.11	1.14
R. 226	2.72	17.7	3.50	1.29
R. 227	3.17	18.9	4.82	1.52
R. 228	3.00	18.5	4.33	1.44
R. 229	3.13	18.8	3.78	1.21
R. 230	2.75	17.8	3.19	1.16

TABLES III: 1—III: 15

Islet tissue analysis in individual animals

TABLE III: I

Group I A

Animal no.		l	k	I mm²	N	M mm²	n_i	I_c mm²	i mm²	V_i mm³	V_i/BW mm³	n_i/BW	m_i μ^2	n_c
R. 101	D	6	6	122,477	406	292.7	1,158	338,932	3.31	3.97	1.86	922	3,202	36,000
	L	4	4	126,110	333	378.0	816	308,412	3.01	3.61	1.69			
	DL	10	10	248,587	739	327.9	1,974	647,344	6.32	7.58	3.54			
R. 103	D	7	7	150,802	329	411.2	1,009	414,911	4.05	4.86	1.95	865	4,044	34,900
	L	5	5	134,365	339	416.2	1,145	476,579	4.65	5.58	2.24			
	DL	12	11	285,167	668	413.9	2,154	891,490	8.71	10.45	4.20			
R. 106	D	4	3	121,326	282	409.0	761	311,269	3.04	3.65	1.44	710	4,335	28,300
	L	4	3	147,767	313	469.4	1,036	486,309	4.75	5.70	2.25			
	DL	8	6	269,093	595	443.8	1,797	797,578	7.79	9.35	3.70			
R. 108	D	4	3	112,781	314	352.6	707	249,291	2.43	2.92	1.92	922	3,274	25,300
	L	4	2	82,620	260	317.3	695	220,497	2.15	2.58	1.70			
	DL	8	5	195,401	574	335.1	1,402	469,788	4.59	5.51	3.63			
R. 122	D	4	2	84,944	261	325.8	1,099	358,009	3.50	4.20	2.90	1,503	2,908	42,200
	L	4	2	72,909	266	269.8	1,081	291,651	2.85	3.42	2.36			
	DL	8	4	157,853	527	298.0	2,180	649,660	6.34	7.61	5.25			
R. 129	D	5	2	84,108	256	328.1	890	292,000	2.85	3.42	1.97	934	3,561	28,200
	L	3	2	103,296	260	409.2	736	301,140	2.94	3.53	2.03			
	DL	8	4	187,404	516	364.8	1,626	593,140	5.79	6.95	3.99			
R. 134	D	10	4	113,514	310	357.1	1,154	412,120	4.02	4.82	1.77	810	3,571	37,900
	L	6	3	112,743	289	374.7	1,050	393,466	3.84	4.61	1.69			
	DL	16	7	226,257	599	365.5	2,204	805,586	7.87	9.44	3.47			
R. 136	D	7	4	115,723	305	380.9	919	350,079	3.42	4.10	1.81	1,007	3,348	40,900
	L	7	[illegible]	97,484	295	316.8	1,366	432,801	4.23	5.08	2.24			

TABLE III: 2

Group IV B

TABLE III: 2

Group IV B

Animal no.		l	k	I mm²	N	M mm²	n_i	I_c mm²	i mm²	V_i mm³	V_i/BW mm³	n_i/BW	m_i μ^2	n_c
R. 102	D	5	5	95,394	373	258.9	609	157,681	1.54	1.85	0.80			
	L	4	4	107,485	365	304.8	760	231,614	2.26	2.71	1.18	595	2,776	26,900
	DL	9	9	202,879	738	284.4	1,369	389,295	3.80	4.56	1.98			
R. 104	D	4	4	108,659	308	330.8	582	192,515	1.88	2.26	0.99			
	L	3	3	113,245	318	364.2	800	291,420	2.85	3.42	1.50	606	3,423	24,400
	DL	7	7	221,904	626	350.2	1,382	483,935	4.73	5.68	2.49			
R. 107	D	6	3	108,221	302	357.6	855	305,766	2.99	3.59	1.25			
	L	4	3	125,310	312	398.7	955	380,733	3.72	4.46	1.55	628	3,702	30,600
	DL	10	6	233,531	614	379.3	1,810	686,499	6.70	8.04	2.79			
R. 109	D	4	2	82,490	252	320.6	510	163,486	1.60	1.92	0.73			
	L	4	3	97,567	316	331.4	824	273,066	2.67	3.20	1.22	507	3,193	24,300
	DL	8	5	180,057	568	327.3	1,334	436,552	4.26	5.11	1.94			
R. 123	D	6	5	79,656	285	278.5	1,051	292,676	2.86	3.43	1.73			
	L	2	2	89,109	249	363.3	692	251,407	2.46	2.95	1.49	880	3,046	32,200
	DL	8	7	168,765	534	312.2	1,743	544,083	5.31	6.37	3.22			
R. 133	D	7	3	92,727	292	329.9	931	307,155	3.00	3.60	1.39			
	L	3	2	65,120	255	259.2	447	115,868	1.13	1.36	0.53	532	2,997	26,000
	DL	10	5	157,847	547	307.0	1,378	423,023	4.13	4.96	1.92			
R. 135	D	6	2	70,062	250	277.4	401	111,251	1.09	1.31	0.44			
	L	3	3	95,849	292	305.7	675	206,378	2.02	2.42	0.81	361	2,881	20,800
	DL	9	5	165,911	542	295.2	1,076	317,629	3.10	3.72	1.25			
R. 137	D	8	4	84,545	313	257.2	979	251,838	2.46	2.95	1.24			
	L	3	2	83,905	268	309.3	526	162,710	1.59	1.91	0.80	632	2,691	29,900
	DL	11	6	168,450	581	275.4	1,505	414,548	4.05	4.86	2.04			

TABLE III: 3 Group II A

Animal no.		l	k	I mm²	N	M mm²	n_i	I_c mm²	i mm²	V_i mm³	V_i/BW mm³	n_i/BW	m_i μ^2	n_c
R. 113	D	10	5	59,695	163	367.5	2,560	940,737	9.19	11.03	4.39			
	L	2	2	33,627	88	367.0	1,057	387,924	3.79	4.55	1.81	1,441	3,589	62,000
	DL	12	7	93,322	251	367.3	3,617	1,328,661	12.98	15.58	6.21			
R. 114	D	6	6	52,580	117	452.5	838	379,219	3.70	4.44	2.72			
	L	4	4	45,975	113	408.5	946	386,406	3.77	4.52	2.77	1,094	4,193	28,500
	DL	10	10	98,555	230	429.2	1,784	765,625	7.48	8.98	5.51			
R. 116	D	14	7	63,340	175	366.0	1,799	658,398	6.43	7.72	3.20			
	L	10	5	87,985	186	496.1	1,879	932,153	9.10	10.92	4.53	1,526	4,222	58,200
	DL	24	12	151,325	361	432.4	3,678	1,590,551	15.53	18.64	7.73			
R. 125	D	9	4	25,940	87	305.4	1,052	321,268	3.14	3.77	1.35			
	L	4	3	32,265	97	335.9	1,187	398,674	3.89	4.67	1.67	800	3,140	41,500
	DL	13	7	58,205	184	321.5	2,239	719,942	7.03	8.44	3.01			
R. 126	D	8	4	34,795	110	331.5	1,593	528,057	5.16	6.19	2.18			
	L	5	2	28,475	85	330.3	1,226	404,967	3.95	4.74	1.67	993	3,232	51,200
	DL	13	6	63,270	195	331.0	2,819	933,024	9.11	10.93	3.85			
R. 156	D	7	3	26,945	72	384.9	1,029	396,028	3.87	4.64	1.87			
	L	4	4	52,871	119	454.9	837	380,775	3.72	4.46	1.80	752	4,068	30,200
	DL	11	7	79,816	191	416.3	1,866	776,803	7.59	9.11	3.67			
R. 160	D	7	4	37,261	97	388.0	631	244,814	2.39	2.87	1.17			
	L	5	4	47,689	124	383.7	845	324,265	3.17	3.80	1.55	602	3,767	24,800
	DL	12	8	84,950	221	385.6	1,476	569,079	5.56	6.67	2.72			
R. 166	D	6	3	23,960	81	312.6	971	303,500	2.96	3.55	1.47			
	L	5	3	36,961	114	327.8	1,235	404,842	3.95	4.74	1.96	912	3,137	40,400
	DL	11	6	60,921	195	321.1	2,206	708,342	6.92	8.30	3.43			
R. 213	D	9	4	40,755	122	337.3	1,440	485,746	4.74	5.69	2.42			
	L	4	4	69,495	139	505.3	1,041	525,991	5.14	6.17	2.63	1,056	3,982	40,600
	DL	13	8	110,250	261	407.8	2,481	1,011,737	9.88	11.86	5.05			
R. 214	D	10	5	42,135	115	388.3	1,472	571,621	5.58	6.70	2.69			
	L	5	4	78,790	147	539.0	1,297	699,032	6.83	8.20	3.29	1,112	4,482	42,700
	DL	15	9	120,925	262	458.9	2,769	1,270,653	12.41	14.89	5.98			

TABLE III: 4

Group IV C

Animal no.		l	k	I mm^2	N	M mm^2	n_i	I_c mm^2	i mm^2	V_i mm^3	Vi/BW mm^3	n_i/BW	m_i μ^2	n_c
R. 157	D	10	5	45,595	147	303.3	1,211	367,287	3.59	4.31	1.48	534	3,057	28,900
	L	2	2	20,990	59	346.5	343	118,859	1.16	1.39	0.48			
	DL	12	7	66,585	206	312.8	1,554	486,146	4.75	5.70	1.96			
R. 161	D	5	3	37,535	90	431.7	755	325,942	3.18	3.82	1.34	648	3,835	30,500
	L	4	3	38,230	109	366.1	1,091	399,445	3.90	4.68	1.64			
	DL	9	6	75,765	199	393.0	1,846	725,387	7.08	8.50	2.98			
R. 115	D	8	3	13,200	67	196.6	783	153,930	1.50	1.80	0.80	692	2,025	35,800
	L	5	4	26,215	121	217.8	768	167,238	1.63	1.96	0.88			
	DL	13	7	39,415	188	207.1	1,551	321,168	3.14	3.77	1.68			
R. 127	D	9	4	28,720	102	283.6	1,400	397,002	3.88	4.66	1.39	634	2,886	40,300
	L	3	2	22,680	78	318.1	731	232,538	2.27	2.72	0.81			
	DL	12	6	51,400	180	295.4	2,131	629,540	6.15	7.38	2.20			

TABLE III: 5

Group IV E

Animal no.		l	k	I mm^2	N	M mm^2	n_i	I_c mm^2	i mm^2	V_i mm^3	Vi/BW mm^3	n_i/BW	m_i μ^2	n_c
R. 169	D	5	5	32,020	110	294.5	540	159,046	1.55	1.86	0.85	536	3,212	21,600
	L	3	3	30,372	85	357.2	640	228,596	2.23	2.68	1.22			
	DL	8	8	62,392	195	328.5	1,180	387,642	3.79	4.55	2.07			
R. 170	D	6	6	47,105	170	279.9	922	258,050	2.52	3.02	1.28	685	2,692	32,300
	L	3	3	22,105	83	270.4	694	187,687	1.83	2.20	0.93			
	DL	9	9	69,210	253	275.8	1,616	445,737	4.35	5.22	2.21			
R. 171	D	6	6	40,457	128	322.5	823	265,395	2.59	3.11	1.33	596	3,290	25,200
	L	3	3	26,954	75	358.3	572	204,925	2.00	2.40	1.03			
	DL	9	9	67,411	203	337.1	1,395	470,320	4.59	5.51	2.35			
R. 172	D	4	4	30,200	85	352.5	737	259,800	2.54	3.05	1.34	626	3,665	24,300
	L	3	3	36,245	91	399.7	690	275,797	2.69	3.23	1.42			
	DL	7	7	66,445	176	375.3	1,427	535,597	5.23	6.28	2.75			

TABLE III: 6

Group IV D

Animal no.		l	k	I mm²	N	M mm²	n_i	I_c mm²	i mm²	V_i mm³	V_i/BW mm³	ni/BW	m_i μ^2	n_c
R. 152	D	7	3	26,520	91	303.6	671	203,690	1.99	2.39	0.84	480	3,057	25,600
	L	4	3	30,190	97	322.1	698	224,852	2.20	2.64	0.93			
	DL	11	6	56,710	188	313.0	1,369	428,542	4.18	5.02	1.76			
R. 153	D	6	3	22,610	78	299.3	840	251,425	2.46	2.95	1.13	610	3,406	28,100
	L	4	4	48,185	119	403.9	753	304,156	2.97	3.56	1.37			
	DL	10	7	70,795	197	348.8	1,593	555,581	5.43	6.51	2.50			
R. 154	D	10	5	50,390	124	426.0	852	362,911	3.54	4.25	1.21	441	4,473	24,100
	L	4	2	31,270	63	496.9	704	349,834	3.42	4.10	1.16			
	DL	14	7	81,660	187	458.1	1,556	712,745	6.96	8.35	2.37			
R. 155	D	8	4	29,085	100	296.7	855	253,715	2.48	2.97	0.91	558	3,458	31,900
	L	4	2	34,510	85	404.9	965	390,763	3.82	4.58	1.40			
	DL	12	6	63,595	185	354.1	1,820	644,478	6.29	7.55	2.32			

TABLE III: 7

Group IV A

Animal no.		l	k	I mm²	N	M mm²	n_i	I_c mm²	i mm²	V_i mm³	V_i/BW mm³	n_i/BW	m_i μ^2	n_c
R. 178	D	7	3	35,620	89	407.4	1,106	450,542	4.40	5.28	1.65			
	L	5	4	52,800	132	396.2	1,007	399,012	3.90	4.68	1.46	660	3,928	34,500
	DL	12	7	88,420	221	402.1	2,113	849,554	8.30	9.96	3.11			
R. 179	D	6	4	36,255	93	440.0	844	371,334	3.63	4.36	1.44			
	L	3	3	54,010	112	471.8	791	373,226	3.64	4.37	1.45	541	4,446	25,200
	DL	9	7	90,265	205	455.4	1,635	744,560	7.27	8.72	2.89			
R. 241	D	9	4	34,315	103	330.1	1,075	354,857	3.47	4.16	1.56			
	L	6	3	34,975	107	326.8	1,062	347,102	3.39	4.07	1.52	800	3,210	38,800
	DL	15	7	69,290	210	328.5	2,137	701,959	6.86	8.23	3.08			
R. 242	D	9	4	30,900	93	332.7	904	300,748	2.94	3.53	1.08			
	L	9	4	52,205	153	331.6	1,497	496,433	4.85	5.82	1.77	732	3,242	43,400
	DL	18	8	83,105	246	332.0	2,401	797,181	7.78	9.34	2.85			

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TABLE III: 8

Group IV F a

Animal no.		l	k	I mm²	N	M mm²	n_i	I_c mm²	i mm²	V_i mm³	V_i/BW mm³	n_i/BW	m_i μ^2	n_c
R. 225	D	9	5	108,070	220	479.2	2,652	1,270,876	12.41	14.89	5.46			
	L	5	4	120,295	233	519.4	1,805	937,457	9.15	10.99	4.02	1,633	4,839	66,000
	DL	14	9	228,365	453	495.5	4,457	2,208,333	21.57	25.88	9.48			
R. 226	D	7	4	32,420	131	249.5	1,289	321,657	3.14	3.77	1.39			
	L	5	2	17,640	71	257.8	846	218,134	2.13	2.56	0.94	785	2,469	44,600
	DL	12	6	50,060	202	252.8	2,135	539,791	5.27	6.33	2.33			
R. 227	D	8	4	50,115	130	391.0	1,379	539,178	5.27	6.32	1.99			
	L	6	3	44,564	104	428.9	1,573	674,629	6.59	7.91	2.49	931	4,015	47,900
	DL	14	7	94,679	234	411.2	2,952	1,213,807	11.85	14.22	4.49			
R. 228	D	8	4	31,400	105	308.1	1,313	404,496	3.95	4.74	1.58			
	L	6	3	41,910	114	368.7	1,414	521,272	5.09	6.11	2.04	909	3,315	48,900
	DL	14	7	73,310	219	339.5	2,727	925,768	9.04	10.85	3.62			
R. 229	D	8	4	71,545	149	460.5	1,863	857,882	8.38	10.05	3.21			
	L	6	3	69,725	171	406.4	2,136	868,102	8.48	10.17	3.25	1,278	4,215	63,400
	DL	14	7	141,270	320	431.6	3,999	1,725,984	16.86	20.23	6.46			
R. 230	D	8	4	53,475	148	349.8	1,620	566,596	5.53	6.64	2.41			
	L	4	3	65,735	170	391.8	1,135	444,664	4.34	5.21	1.89	1,002	3,585	47,400
	DL	12	7	119,210	318	367.1	2,755	1,011,260	9.88	11.85	4.31			

TABLE III: 9

Group I B

Animal no.		l	k	I mm²	N	M mm²	n_i	I_c mm²	i mm²	V_i mm³	V_i/BW mm³	n_i/BW	m_i μ^2	n_c
R. 14	D	5	5	34,645	153	217.2	375	81,468	0.80	0.95	0.68			
	L	6	6	67,195	235	274.7	438	120,332	1.18	1.41	1.01	581	2,424	17,000
	DL	11	11	101,840	388	248.2	813	201,800	1.97	2.36	1.69			
R. 193	D	10	5	12,630	102	125.1	657	82,176	0.80	0.96	0.34			
	L	5	3	17,128	95	176.5	648	114,345	1.12	1.34	0.47	461	1,471	35,600
	DL	15	8	29,758	197	150.6	1,305	196,521	1.92	2.30	0.81			
R. 194	D	10	4	21,688	106	200.8	967	194,141	1.90	2.28	0.97			
	L	5	4	31,865	151	208.6	912	190,217	1.86	2.23	0.95	800	1,998	43,700
	DL	15	8	53,553	257	204.6	1,879	384,358	3.75	4.50	1.92			
R. 216	D	11	4	20,022	108	173.0	411	71,113	0.69	0.83	0.35			
	L	8	3	13,345	73	191.6	345	66,104	0.65	0.77	0.32	314	1,772	18,800
	DL	19	7	33,367	181	181.5	756	137,217	1.34	1.61	0.67			
R. 180	D	15	5	24,255	150	160.7	1,186	190,606	1.86	2.23	0.73			
	L	10	5	35,415	183	181.0	1,539	278,580	2.72	3.26	1.07	896	1,681	77,800
	DL	25	10	59,670	333	172.2	2,725	469,186	4.58	5.50	1.81			

Group II B

Animal no.		l	k	I mm²	N	M mm²	n_i	I_c mm²	i mm²	V_i mm³	V_i/BW mm³	n_i/BW	m_i μ^2	n_c
R. 148	D	6	3	16,160	81	206.1	493	101,598	0.99	1.19	0.37			
	L	4	4	31,335	145	222.3	668	148,498	1.45	1.74	0.54	361	2,104	29,800
	DL	10	7	47,495	226	215.4	1,161	250,096	2.44	2.93	0.91			
R. 149	D	7	3	13,601	88	156.0	818	127,634	1.25	1.50	0.47			
	L	5	4	25,075	128	195.5	783	153,083	1.50	1.79	0.57	508	1,712	40,700
	DL	12	7	38,676	216	175.3	1,601	280,717	2.74	3.29	1.04			
R. 174	D	6	4	19,895	146	137.8	595	82,015	0.80	0.96	0.34			
	L	3	3	18,025	128	140.7	634	89,210	0.87	1.05	0.37	434	1,361	35,400
	DL	9	7	37,920	274	139.3	1,229	171,225	1.67	2.01	0.71			

TABLE III: 11

Group IV G a

Animal no.		l	k	I mm²	N	M mm²	n_i	I_c mm²	i mm²	V_i mm³	V_i/BW mm³	n_i/BW	m_i μ^2	n_c
R. 143	D	4	2	8,110	64	123.4	219	27,016	0.26	0.32	0.13			
	L	5	3	13,140	102	125.4	438	54,945	0.54	0.64	0.26	264	1,218	20,600
	DL	9	5	21,250	166	124.8	657	81,961	0.80	0.96	0.39			
R. 146	D	11	5	14,185	83	173.0	353	61,080	0.60	0.72	0.21			
	L	4	3	12,410	78	159.8	349	55,770	0.54	0.65	0.19	208	1,625	18,200
	DL	15	8	26,595	161	166.5	702	116,850	1.14	1.37	0.41			
R. 147	D	7	3	11,910	92	136.7	434	59,339	0.58	0.70	0.23			
	L	5	4	15,950	101	159.7	522	83,360	0.81	0.98	0.32	311	1,458	26,400
	DL	12	7	27,860	193	149.3	956	142,699	1.39	1.67	0.54			
R. 165	D	7	3	6,730	70	92.6	346	32,049	0.31	0.38	0.10			
	L	5	3	8,475	77	107.8	478	51,529	0.50	0.60	0.17	228	991	28,500
	DL	12	6	15,205	147	101.4	824	83,578	0.82	0.98	0.27			

TABLE III: 12

Group IV G b

Animal no.		l	k	I mm²	N	M mm²	n_i	I_c mm²	i mm²	V_i mm³	V_i/BW mm³	n_i/BW	m_i μ^2	n_c
R. 243	D	8	4	14,525	126	114.3	478	54,628	0.53	0.64	0.18			
	L	6	3	9,400	91	103.8	550	57,075	0.56	0.67	0.19	292	1,061	33,700
	DL	14	7	23,925	217	108.7	1,028	111,703	1.09	1.31	0.37			
R. 245	D	6	3	12,405	71	172.3	405	69,763	0.68	0.82	0.31			
	L	5	3	20,320	120	170.5	446	76,025	0.74	0.89	0.34	322	1,673	21,600
	DL	11	6	32,725	191	171.3	851	145,788	1.42	1.71	0.65			
R. 246	D	8	4	14,070	112	128.0	571	73,096	0.71	0.86	0.21			
	L	7	3	12,980	91	142.0	612	86,935	0.85	1.02	0.25	296	1,321	34,600
	DL	15	7	27,050	203	135.3	1,183	160,031	1.56	1.88	0.47			
R. 247	D	10	3	22,345	96	232.7	686	159,601	1.56	1.87	0.48			
	L	7	3	19,855	92	210.3	611	128,467	1.26	1.51	0.39	334	2,169	28,900
	DL	17	6	42,200	188	222.1	1,297	288,068	2.81	3.38	0.87			
R. 248	D	8	4	11,935	100	116.1	412	47,850	0.47	0.56	0.17			
	L	5	3	11,790	74	159.2	296	47,132	0.46	0.55	0.17	213	1,310	20,900
	DL	13	7	23,725	174	134.2	708	94,982	0.93	1.11	0.33			

Animal no.		l	k	I mm^2	N	M mm^2	n_i	I_c mm^2	i mm^2	V_i mm^3	V_i/BW mm^3	n_i/BW	m_i μ^2	n_c
	D	5	3	29,855	132	204.6	1,041	212,958	2.08	2.50	0.95			
R. 177	L	2	2	18,745	117	163.7	998	163,377	1.60	1.91	0.73	778	1,802	50,500
	DL	7	5	48,600	249	184.6	2,039	376,335	3.68	4.41	1.68			
	D	4	2	12,425	87	140.5	704	98,936	0.97	1.16	0.43			
R. 182	L	3	3	9,360	84	115.6	453	52,386	0.51	0.61	0.23	429	1,277	35,000
	DL	7	5	21,785	171	130.8	1,157	151,322	1.48	1.77	0.66			
	D	5	3	24,240	135	179.0	789	141,241	1.38	1.66	0.55			
R. 183	L	3	2	13,230	92	138.7	816	113,173	1.11	1.33	0.44	537	1,548	43,200
	DL	8	5	37,470	227	158.5	1,605	254,414	2.48	2.98	1.00			
	D	9	4	16,298	116	138.0	1,118	154,267	1.51	1.81	0.64			
R. 205	L	5	2	20,888	133	162.4	1,159	188,217	1.84	2.21	0.78	807	1,469	62,500
	DL	14	6	37,186	249	150.4	2,277	342,484	3.34	4.01	1.42			
	D	10	4	30,250	138	227.5	1,126	256,201	2.50	3.00	1.15			
R. 206	L	4	2	28,905	116	249.8	1,031	257,504	2.51	3.02	1.16	826	2,326	46,700
	DL	14	6	59,155	254	238.2	2,157	513,705	5.02	6.02	2.31			
	D	7	3	27,935	150	186.7	987	184,244	1.80	2.16	0.88			
R. 209	L	4	2	24,495	158	153.7	1,623	249,479	2.44	2.92	1.20	1,070	1,623	67,900
	DL	11	5	52,430	308	166.2	2,610	433,723	4.24	5.08	2.08			
	D	7	3	14,210	126	114.5	809	92,640	0.90	1.09	0.41			
R. 210	L	7	4	25,440	219	123.1	1,759	216,537	2.11	2.54	0.95	962	1,176	80,000
	DL	14	7	39,650	345	120.4	2,568	309,177	3.02	3.62	1.36			
	D	6	3	28,310	160	180.3	1,187	214,065	2.09	2.51	1.00			
R. 185	L	4	3	23,055	168	132.4	1,035	136,986	1.34	1.61	0.64	885	1,543	59,700
	DL	10	6	51,365	328	158.0	2,222	351,051	3.43	4.11	1.64			
	D	8	4	12,620	155	80.8	658	53,143	0.52	0.62	0.18			
R. 186	L	5	3	9,870	129	77.1	1,060	81,774	0.80	0.96	0.27	490	767	69,700
	DL	13	7	22,490	284	78.5	1,718	134,917	1.32	1.58	0.45			
	D	8	4	11,620	116	100.1	1,139	113,977	1.11	1.34	0.34			
R. 191	L	7	3	25,580	203	130.0	1,380	179,429	1.75	2.10	0.53	639	1,138	80,000
	DL	15	7	37,200	319	116.5	2,519	293,406	2.87	3.44	0.87			

TABLE III: 14

Group III B

Animal no.		l	k	I mm²	N	M mm²	n_i	I_c mm²	i mm²	V_i mm³	V_i/BW mm³	n_i/BW	m_i μ^2	n_c
R. 201	D	8	4	11,837	129	89.8	1,120	100,534	0.98	1.18	0.37			
	L	5	3	14,835	149	102.8	1,037	106,647	1.04	1.25	0.40	683	938	77,000
	DL	13	7	26,672	278	96.1	2,157	207,181	2.02	2.43	0.77			
R. 202	D	8	3	10,765	117	93.9	1,354	127,118	1.24	1.49	0.49			
	L	5	3	9,610	107	88.4	1,393	123,147	1.20	1.44	0.47	904	890	101,000
	DL	13	6	20,375	224	91.1	2,747	250,265	2.44	2.93	0.96			
R. 217	D	10	3	4,600	68	62.4	634	39,555	0.39	0.46	0.15			
	L	7	3	10,010	131	77.6	724	56,194	0.55	0.66	0.21	431	689	59,400
	DL	17	6	14,610	199	70.5	1,358	95,749	0.94	1.12	0.36			
R. 218	D	8	3	8,535	108	79.7	713	56,848	0.56	0.67	0.21			
	L	5	3	8,660	116	74.7	625	46,673	0.46	0.55	0.18	430	756	54,800
	DL	13	6	17,195	224	77.4	1,338	103,521	1.01	1.21	0.39			
R. 215	D	7	3	8,905	96	91.3	864	78,863	0.77	0.92	0.27			
	L	6	4	19,015	202	95.8	1,383	132,435	1.29	1.55	0.46	661	918	81,200
	DL	13	7	27,920	298	94.0	2,247	211,298	2.06	2.48	0.73			

TABLE III: 15

Group III C a

Animal no.		l	k	I mm²	N	M mm²	n_i	I_c mm²	i mm²	V_i mm³	V_i/BW mm³	n_i/BW	m_i μ^2	n_c
R. 211	D	7	3	22,300	130	166.0	1,527	253,455	2.48	2.97	1.27			
	L	3	3	23,080	124	186.4	1,063	198,121	1.93	2.32	0.99	1,107	1,703	65,600
	DL	10	6	45,380	254	174.4	2,590	451,576	4.41	5.29	2.26			
R. 212	D	9	4	15,640	136	114.3	1,491	170,424	1.66	2.00	0.67			
	L	5	3	18,085	154	117.9	1,413	166,599	1.63	1.95	0.66	981	1,133	92,400
	DL	14	7	33,725	290	116.1	2,904	337,023	3.29	3.95	1.33			
R. 195	D	10	5	22,885	188	115.8	1,763	204,177	1.99	2.39	0.83			
	L	5	3	28,865	201	141.9	1,634	231,818	2.26	2.72	0.94	1,171	1,253	102,000
	DL	15	8	51,750	389	128.3	3,397	435,995	4.26	5.11	1.76			
R. 196	D	6	3	20,565	96	222.3	1,041	231,444	2.26	2.71	1.15			
	L	5	4	45,720	238	191.6	1,297	248,554	2.43	2.91	1.23	991	2,005	53,900
	DL	11	7	66,285	334	205.3	2,338	479,998	4.69	5.63	2.38			
R. 197	D	10	4	28,015	148	183.1	1,529	279,994	2.73	3.28	1.01			
	L	11	5	30,640	173	183.2	1,831	335,362	3.28	3.93	1.21	1,037	1,789	82,900
	DL	21	9	58,655	321	183.1	3,360	615,356	6.01	7.21	2.23			
R. 198	D	10	5	38,515	235	161.5	2,553	412,275	4.03	4.83	1.60			
	L	5	3	26,505	172	153.4	1,836	281,576	2.75	3.30	1.09	1,453	1,544	117,300
	DL	15	8	65,020	407	158.1	4,389	693,851	6.78	8.13	2.69			
R. 199	D	8	4	27,670	165	167.9	1,064	178,595	1.74	2.09	0.66			
	L	5	3	24,758	105	235.7	987	232,666	2.27	2.73	0.86	645	1,958	48,200
	DL	13	7	52,428	270	200.5	2,051	411,261	4.02	4.82	1.52			
R. 200	D	10	4	19,531	168	115.0	1,392	160,145	1.56	1.88	0.65			
	L	5	3	21,015	156	130.6	1,688	220,405	2.15	2.58	0.89	1,066	1,207	94,500
	DL	15	7	40,546	324	123.6	3,080	380,550	3.72	4.46	1.54			
R. 207	D	10	5	24,105	207	117.4	2,493	292,641	2.86	3.43	1.21			
	L	3	2	23,215	147	158.3	1,260	199,435	1.95	2.34	0.83	1,326	1,280	111,300
	DL	13	7	47,320	354	131.1	3,753	492,076	4.81	5.77	2.04			

TABLES IV: 1—IV: 15

Alpha and beta cell count in individual animals

TABLE IV: 1

Group I A

Animal no.	Part D					Part L					D + L				
	α	β	$\alpha+\beta$	α %	No. of islets	α	β	$\alpha+\beta$	α %	No. of islets	α	β	$\alpha+\beta$	α %	No. of islets
R. 101	82	431	513	15.98	25	137	760	897	15.27	25	219	1,191	1,410	15.53	50
R. 103	82	515	597	13.74	25	152	904	1,056	14.39	25	234	1,419	1,653	14.16	50
R. 106	98	641	739	13.26	25	143	791	934	15.31	25	241	1,432	1,673	14.41	50
R. 108	66	510	576	11.46	25	71	671	742	9.57	25	137	1,181	1,318	10.39	50
R. 122	46	385	431	10.67	25	87	589	676	12.87	25	133	974	1,107	12.01	50
R. 129	101	429	530	19.06	25	207	623	830	24.94	25	308	1,052	1,360	22.65	50
R. 134	85	496	581	14.63	25	67	629	696	9.63	25	152	1,125	1,277	11.90	50
R. 136	50	481	531	9.42	25	39	833	872	4.47	25	89	1,314	1,403	6.34	50
S	610	3,888	4,498	—	200	903	5,800	6,703	—	200	1,513	9,688	11,201	—	400
x̄	—		562	13.56	25	—		838	13.47	25	—		1,400	13.51	50

TABLE IV: 2

Group IV B

Animal no.	Part D					Part L					D + L				
	α	β	$\alpha+\beta$	α %	No. of islets	α	β	$\alpha+\beta$	α %	No. of islets	α	β	$\alpha+\beta$	α %	No. of islets
R. 102	101	545	646	15.63	25	137	369	506	27.08	25	238	914	1,152	20.66	50
R. 104	131	469	600	21.83	25	109	616	725	15.03	25	240	1,085	1,325	18.11	50
R. 107	131	470	601	21.80	25	276	654	930	29.68	25	407	1,124	1,531	26.58	50
R. 109	171	601	772	22.15	25	181	450	631	28.68	25	352	1,051	1,403	25.09	50
R. 123	96	644	740	12.97	25	78	715	793	9.84	25	174	1,359	1,533	11.35	50
R. 133	137	391	528	25.95	25	144	511	655	21.98	25	281	902	1,183	23.75	50
R. 155	86	436	522	16.48	25	226	589	815	27.73	25	312	1,025	1,337	23.34	50
R. 137	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

TABLE IV: 3

Group II A

TABLE IV: 3

Group II A

Animal no.	Part D					Part L					D + L				
	α	β	$\alpha+\beta$	α %	No. of islets	α	β	$\alpha+\beta$	α%	No. of islets	α	β	$\alpha+\beta$	α %	No. of islets
R. 113	49	356	405	12.10	25	150	804	954	15.72	25	199	1,160	1,359	14.64	50
R. 114	96	609	705	13.62	25	57	561	618	9.22	25	153	1,170	1,323	11.56	50
R. 116	121	530	651	18.59	25	216	822	1,039	20.81	25	337	1,352	1,689	19.95	50
R. 125	126	414	540	23.33	25	157	711	868	18.09	25	283	1,125	1,408	20.10	50
R. 126	249	442	691	36.03	25	213	696	909	23.43	25	462	1,138	1,600	28.88	50
R. 156	24	498	522	4.60	25	33	676	709	4.65	25	57	1,174	1,231	4.63	50
R. 160	138	431	569	24.25	25	133	813	946	14.06	25	271	1,244	1,515	17.89	50
R. 166	128	381	509	25.15	25	113	586	699	16.17	25	241	967	1,208	19.95	50
R. 213	117	570	687	17.03	25	121	754	875	13.83	25	238	1,324	1,562	15.24	50
R. 214	106	443	549	19.31	25	185	949	1,134	16.31	25	291	1,392	1,683	17.29	50
R. 219	63	580	643	9.80	25	71	615	686	10.35	25	134	1,195	1,329	10.08	50
S	1,217	5,254	6,471	—	275	1,449	7,987	9,436	—	275	2,666	13,241	15,907	—	550
x̄	—		588	18.81	25	—		858	15.36	25	—		1,446	16.76	50

TABLE IV: 4

Group IV C

Animal no.	Part D					Part L					D + L				
	α	β	$\alpha+\beta$	α %	No. of islets	α	β	$\alpha+\beta$	α %	No. of islets	α	β	$\alpha+\beta$	α %	No. of islets
R. 157	71	574	645	11.01	25	72	710	782	9.21	25	143	1,284	1,427	10.02	50
R. 161	108	576	684	15.79	25	128	687	815	15.71	25	236	1,263	1,499	15.74	50
R. 115	94	356	450	20.89	25	203	900	1,103	18.40	50	297	1,256	1,553	19.12	75
R. 127	92	368	460	20.00	25	111	563	674	16.47	25	203	931	1,134	17.90	50
S	365	1,874	2,239	—	100	514	2,860	3,374	—	125	879	4,734	5,613	—	225
x̄	—		560	16.30	25	—		844	15.23	31	—		1,403	15.66	56

TABLE IV: 5

Group IV E

Animal no.	Part D					Part L					D + L				
	α	β	α+β	α %	No. of islets	α	β	α+β	α %	No. of islets	α	β	α+β	α %	No. of islets
R. 169	137	471	608	22.53	25	73	551	624	11.70	25	210	1,022	1,232	17.05	50
R. 170	208	537	745	27.92	25	185	412	597	30.99	25	393	949	1,342	29.28	50
R. 171	92	427	519	17.73	26	147	549	696	21.12	26	239	976	1,215	19.67	52
R. 172	90	529	619	14.54	25	130	896	1,026	12.67	25	220	1,425	1,645	13.37	50
S	527	1,964	2,491	—	101	535	2,408	2,943	—	101	1,062	4,372	5,434	—	202
x̄	—		623	21.16	25	—		736	18.18	25	—		1,359	19.54	51

TABLE IV: 6

Group IV D

Animal no.	Part D					Part L					D + L				
	α	β	α+β	α %	No. of islets	α	β	α+β	α%	No. of islets	α	β	α+β	α %	No. of islets
R. 152	130	402	532	24.44	25	148	583	731	20.25	25	278	985	1,263	22.01	50
R. 153	154	546	700	22.00	25	149	662	811	18.37	25	303	1,208	1,511	20.05	50
R. 154	171	566	737	23.20	25	203	1,151	1,354	14.99	25	374	1,717	2,091	17.89	50
R. 155	98	522	620	15.81	25	194	937	1,131	17.15	25	292	1,459	1,751	16.68	50
S	553	2,036	2,589		100	694	3,333	4,027	—	100	1,247	5,369	6,616	—	200
x̄	—		647	21.36	25	—		1,007	17.23	25	—		1,654	18.85	50

TABLE IV: 7

Group IV A

Animal no.	Part D					Part L					D + L				
	α	β	$\alpha+\beta$	α %	No. of islets	α	β	$\alpha+\beta$	α %	No. of islets	α	β	$\alpha+\beta$	α %	No. of islets
R. 178	166	552	718	23.12	25	160	438	598	26.76	25	326	990	1,316	24.77	50
R. 179	222	782	1,004	22.11	25	211	944	1,155	18.27	25	433	1,726	2,159	20.06	50
R. 241	152	548	700	21.71	25	165	660	825	20.00	25	317	1,208	1,525	20.79	50
R. 242	100	531	631	15.85	25	128	729	857	14.94	25	228	1,260	1,488	15.32	50
S	640	2,413	3,053	—	100	664	2,771	3,435	—	100	1,304	5,184	6,488	—	200
$\bar{x}$	—		763	20.96	25	—		859	19.33	25	—		1,622	20.10	50

TABLE IV: 8

Group IV F a

Animal no.	Part D					Part L					D + L				
	α	β	$\alpha+\beta$	α %	No. of islets	α	β	$\alpha+\beta$	α %	No. of islets	α	β	$\alpha+\beta$	α %	No. of islets
R. 225	29	689	718	4.04	25	120	1,113	1,233	9.73	25	149	1,802	1,951	7.64	50
R. 226	191	615	806	23.70	50	131	483	614	21.34	25	322	1,098	1,420	22.68	75
R. 227	99	436	535	18.50	25	182	448	630	28.89	25	281	884	1,165	24.12	50
R. 228	99	538	637	15.54	25	131	452	583	22.47	25	230	990	1,220	18.85	50
R. 229	100	566	666	15.02	25	134	751	885	15.14	25	234	1,317	1,551	15.09	50
R. 230	75	469	544	13.79	25	100	670	770	12.99	25	175	1,139	1,314	13.32	50
S	593	3,313	3,906	—	175	798	3,917	4,715	—	150	1,391	7,230	8,621	—	325
$\bar{x}$	—		651	15.18	29	—		786	16.92	25	—		1,437	16.14	54

TABLE IV: 9

Group I B

Animal no.	Part D					Part L					D + L				
	α	β	$\alpha+\beta$	α %	No. of islets	α	β	$\alpha+\beta$	α%	No. of islets	α	β	$\alpha+\beta$	α %	No. of islets
R. 194	79	284	363	21.76	25	219	642	861	25.44	50	298	926	1,224	24.35	75
R. 216	217	207	424	51.18	25	408	350	758	53.83	50	625	557	1,182	52.88	75
R. 180	249	54	303	82.18	25	745	175	920	80.98	50	994	229	1,223	81.28	75
S	545	545	1,090	—	75	1,372	1,167	2,539	—	150	1,917	1,712	3,629	—	225
$\bar{x}$	—		363	50.00	25	—		846	54.04	50	—		1,210	52.82	75

TABLE IV: 10

Group II B

Animal no.	Part D					Part L					D + L				
	α	β	$\alpha+\beta$	α %	No. of islets	α	β	$\alpha+\beta$	α %	No. of islets	α	β	$\alpha+\beta$	α %	No. of islets
R. 148	230	235	465	49.46	25	365	541	906	40.29	50	595	776	1,371	43.40	75
R. 149	177	188	365	48.49	25	399	480	879	45.39	50	576	668	1,244	46.30	75
R. 174	131	205	336	38.99	25	252	488	740	34.05	50	383	693	1,076	35.59	75
S	538	628	1,166	—	75	1,016	1,509	2,525	—	150	1,554	2,137	3,691	—	225
$\bar{x}$			389	46.14	25	—		842	40.24	50	—		1,230	42.10	75

TABLE IV: 11

Group IV G a

Animal no.	Part D					Part L					D + L				
	α	β	$\alpha+\beta$	α %	No. of islets	α	β	$\alpha+\beta$	α %	No. of islets	α	β	$\alpha+\beta$	α %	No. of islets
R. 143	334	329	663	50.38	50	347	290	637	54.47	50	681	619	1,300	52.38	100
R. 146	287	278	565	50.80	50	365	283	648	56.33	50	652	561	1,213	53.75	100
R. 147	403	223	626	64.38	50	426	112	538	79.18	50	829	335	1,164	71.22	100
R. 165	290	99	389	74.55	50	452	203	655	69.01	75	742	302	1,044	71.07	125
S	1,314	929	2,243	—	200	1,590	888	2,478	—	225	2,904	1,817	4,721	—	425
$\bar{x}$	—		561	58.58	50	—		620	64.16	56	—		1,180	61.51	106

TABLE IV: 12

Group IV G b

Animal no.	Part D					Part L					D + L				
	α	β	$\alpha+\beta$	α %	No. of islets	α	β	$\alpha+\beta$	α %	No. of islets	α	β	$\alpha+\beta$	α %	No. of islets
R. 243	350	226	576	60.76	50	458	139	597	76.72	50	808	365	1,173	68.88	100
R. 245	264	50	314	84.08	25	717	144	861	83.28	50	981	194	1,175	83.49	75
R. 246	294	60	354	83.05	25	615	131	746	82.44	50	909	191	1,100	82.64	75
R. 247	293	152	445	65.84	25	445	315	760	58.55	50	738	467	1,205	61.24	75
R. 248	351	47	398	88.19	25	665	74	739	89.99	50	1,016	121	1,137	89.36	75
S	1,552	535	2,087	—	150	2,900	803	3,703	—	250	4,452	1,338	5,790	—	400
$\bar{x}$	—		417	74.37	30	—		741	78.31	50	—		1,158	76.89	80

TABLE IV: 13

Group III A

Animal no.	Part D					Part L					D + L				
	α	β	$\alpha+\beta$	α %	No. of islets	α	β	$\alpha+\beta$	α %	No. of islets	α	β	$\alpha+\beta$	α %	No. of islets
R. 177	69	308	377	18.30	25	247	1,278	1,525	16.20	100	316	1,586	1,902	16.61	125
R. 182	48	253	301	15.95	25	161	822	983	16.38	100	209	1,075	1,284	16.28	125
R. 183	76	253	329	23.10	25	265	1,180	1,445	18.34	100	341	1,433	1,774	19.22	125
R. 205	152	508	660	23.03	50	118	438	556	21.22	50	270	946	1,216	22.20	100
R. 206	71	443	514	13.81	25	136	911	1,047	12.99	50	207	1,354	1,561	13.26	75
R. 209	105	504	609	17.24	50	110	527	637	17.27	50	215	1,031	1,246	17.26	100
R. 210	99	360	459	21.57	50	175	593	768	22.79	75	274	953	1,227	22.33	125
R. 185	67	232	299	22.41	25	194	544	738	26.29	50	261	776	1,037	25.17	75
R. 186	178	552	730	24.38	75	215	319	534	40.26	75	393	871	1,264	31.09	150
R. 191	62	444	506	12.25	50	144	421	565	25.49	50	206	865	1,071	19.23	100
S	927	3,857	4,784	—	400	1,765	7,033	8,798	—	700	2,692	10,890	13,582	—	1,100
x̄	—		478	19.38	40	—		880	20.06	70	—		1,358	19.82	110

TABLE IV: 14

Group III B

Animal no.	Part D					Part L					D + L				
	α	β	$\alpha+\beta$	α %	No. of islets	α	β	$\alpha+\beta$	α %	No. of islets	α	β	$\alpha+\beta$	α %	No. of islets
R. 201	432	358	790	54.68	75	229	383	612	37.42	75	661	741	1,402	47.15	150
R. 202	451	391	842	53.56	75	386	332	718	53.76	75	837	723	1,560	53.65	150
R. 217	349	343	692	50.43	75	307	275	582	52.75	75	656	618	1,274	51.49	150
R. 218	453	350	803	56.41	75	444	213	657	67.58	75	897	563	1,460	61.44	150
R. 215	384	340	724	53.04	75	369	264	633	58.29	75	753	604	1,357	55.49	150
S	2,069	1,782	3,851	—	375	1,735	1,467	3,202	—	375	3,804	3,249	7,053	—	750
x̄	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

TABLE IV: 15

Group III C a

Animal no.	Part D					Part L					D + L				
	α	β	$\alpha+\beta$	α %	No. of islets	α	β	$\alpha+\beta$	α%	No. of islets	α	β	$\alpha+\beta$	α %	No. of islets
R. 211	231	621	852	27.11	50	152	451	603	25.21	50	383	1,072	1,455	26.32	100
R. 212	117	563	680	17.21	100	113	622	735	15.37	100	230	1,185	1,415	16.25	200
R. 195	256	374	630	40.63	75	281	380	661	42.51	75	537	754	1,291	41.60	150
R. 196	313	287	600	52.17	50	252	238	490	51.43	50	565	525	1,090	51.83	100
R. 197	106	387	493	21.50	50	131	417	548	23.91	50	237	804	1,041	22.77	100
R. 198	139	393	532	26.13	50	232	435	667	34.78	75	371	828	1,199	30.94	125
R. 199	260	332	592	43.92	50	259	390	649	39.91	50	519	722	1,241	41.82	100
R. 200	274	443	717	38.21	75	332	310	642	51.71	74	606	753	1,359	44.59	149
R. 207	216	461	677	31.91	75	185	444	629	29.41	75	401	905	1,306	30.70	150
S	1,912	3,861	5,773	—	575	1,937	3,687	5,624	—	599	3,849	7,548	11,397	—	1,174
$\bar{x}$	—		641	33.12	64	—		625	34.44	67	—		1,266	33.77	130

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The Histopathologic Changes in the Myenteric Plexus of the Pylorus in Hypertrophic Pyloric Stenosis of Infants (Pylorospasm)

BY

HILMA ALAROTU

Almqvist & Wiksells Boktryckeri AB UPPSALA

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FROM THE INSTITUTE OF FORENSIC MEDICINE, UNIVERSITY OF HELSINKI (CHIEF: PROFESSOR UNTO UOTILA, M.D.), THE CHILDREN'S CLINIC, UNIVERSITY OF HELSINKI (CHIEF: PROFESSOR ARVO YLPPÖ, M.D.), THE DEPARTMENT OF PATHOLOGY, UNIVERSITY OF HELSINKI (CHIEF AT THE TIME OF THE INVESTIGATION: THE LATE PROFESSOR A. SAXEN, M.D.), AND THE DEPARTMENT OF PATHOLOGY, UNIVERSITY OF COPENHAGEN (CHIEF: PROFESSOR J. ENGELBRETH-HOLM, M.D.)

THE HISTOPATHOLOGIC CHANGES IN THE MYENTERIC PLEXUS OF THE PYLORUS IN HYPERTROPHIC PYLORIC STENOSIS OF INFANTS (PYLOROSPASM)

BY

HILMA ALAROTU

HELSINKI 1956

HELSINKI 1956
MERCATORIN KIRJAPAINO

PREFACE

I am happy to have the opportunity to express my deedgratitude to my highly esteemed teacher, the Physician in Charge of the Children's Clinic of the University of Helsinki, Professor Arvo Ylppö, M.D., Archiater, who has kindly placed the material for my work at my disposal in his hospital and given me the benefit of his valuable criticism during the course of the work.

The object of the present investigation was suggested by the Chief of the Second Pediatric Clinic, Professor C.-E. Räihä, M.D., in 1945. Throughout my work he has followed it with keen interest and aided me with inspiring advice in its various phases, for which my respectful thanks are due to him.

I wish to express my particularly sincere gratitude to the Chief of the Institute of Forensic Medicine of the University of Helsinki, Professor Unto Uotila, M.D., who has in person directed and supervised my prolonged work. His valuable advice has been of very great significance in my work.

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Helsinki, March 1955.

Hilma Alarotu.

CONTENTS

I. INTRODUCTION

Hypertrophic pyloric stenosis of infants was first described by Hirschsprung in 1888. According to him it is a tumorous, primary muscular hypertrophy in which, furthermore, a remarkable growth of connective tissue is observable. Heile (1921) also reached similar conclusions. However, in the opinion of several investigators (Hertz 1916, Wollstein 1922, Priesel 1928, Màtyàs 1932, Donovan 1946, Ladd et al. 1947) only hypertrophy of the muscle layer is involved, the rest of the tissue having a normal appearance. According to other authors (Wernstedt 1907, Hutchison 1910, Careddu and Nicoli 1932) an increase in the muscle cells in number as well as in volume can be established. It has further been observed (Careddu and Nicoli 1932) that at the point where the pylorus terminates and the duodenum begins the fibers of both muscle layers almost wholly lose their characteristic arrangement. The muscle fibers of the circular layer are traversed obliquely by thinner bundles of muscle fibers, which originate from the longitudinal muscle layer or from the muscularis mucosae.

In his investigation on congenital hypertrophic pyloric stenosis, Niero (1947) reached the conclusion that hypertrophy and hyperplasia are observable in all types of tissue: in the circular and longitudinal muscle layers, the connective tissue, the nervous tissue and the mucous membrane. With regard to the longitudinal muscle layer he has established that at the point of transition from the pylorus to the duodenum, the outermost fibers, comprising about one third of the longitudinal muscle layer, continue in their normal course, constituting the corresponding layer of the duodenum, while the bundles in the middle assume a vortical arrangement and those in the deepest parts traverse the circular layer, passing obliquely into the submucosa. These oblique bundles are accompanied by strongly developed connective tissue originating from the longi-

tudinal layer and travelling to the submucosa together with numerous blood vessels, ganglia and nerve fibers. After an investigation of the pylorus of fetuses from the fourth intrauterine month up to birth, he has found that the course of the longitudinal muscle fibers in the pyloric canal of children affected with pyloric stenosis resembles, in the sphincteral part, the course of the muscle fibers in the corresponding part of the fetal pylorus. On the basis of his studies, the said author has come to the conclusion that the disease is referable to two simultaneously acting factors: a spastic factor and a factor producing malformation. The latter is caused by a stasis of development in the antral part, which retains a typically fetal structure, and by local hyperplasia and hypertrophy of all types of tissue. These are brought about by certain changes in the nervous system.

In order to determine the congenital nature of the muscular hypertrophy, Wallgren (1946) examined the stomachs of 1000 newborn boys at the age of 4—7 days and found them to be normal in every respect. At the age of three weeks 5 of the infants examined began to vomit, and the x-ray investigation revealed hypertrophic pyloric stenosis. Accordingly the muscular hypertrophy was not congenital but developed with surprising rapidity and with its typical symptoms shortly before the manifestation of the disease.

In his explanation of hypertrophic pyloric stenosis, Wernstedt (1907) has advanced the spasm theory. Pyloric stenosis of infants, according to him, is a kind of neurosis, caused by the primary, spastic occlusion of the antral part of the stomach adjacent to the duodenum, and a simultaneous secondary muscular hypertrophy developed in the greater part of the stomach.

In further explanation of the origin of the spasm, it has been suggested (Thomson 1902) that it is the result of incoordination of the pyloric mechanism, which is possibly initiated during intrauterine life. According to this view the pylorus closes when the peristaltic waves reach upon it, instead of opening as it should do. Reference has also been made (Shattock 1909) to hyperesthesia of the mucous membrane in the pyloric region, which gives rise to a reflex contraction (»hysterical» pylorus).

Some investigators consider the spasm a disturbance in the chemical mechanism of the pylorus. They state that hyperacidity of the stomach contents exists in these cases and that this hyper-

acidity is spasmogenic (Engel 1909, Pfaundler 1909). On the other hand, it has been claimed (Miller and Willcox 1907, Salmi 1937) that no hyperacidity exists in such cases or, if it occurs, that it is a consequence of the obstruction, not its cause (Feer 1909). Moreover, it has been established experimentally that acid on the gastric side causes opening, and not closing, of the pyloric sphincter (Cannon 1907).

An allergic condition accompanying pylorospasm is indicated by the fact that in the follow-up examination of these children (Salmi 1941) one third of the cases have been found to exhibit skin manifestations of exudative diathesis, such as are encountered in Child Welfare Clinics, on an average, in only one quarter of the children.

Studies of the incidence of pylorospasm according to season (Räihä and Ylppö 1939) have revealed that it occurs with considerably higher frequency in the spring than at other seasons, which would seem to indicate a catarrhal process in the stomach walls as an etiologic factor. At the same time the hypothesis has been advanced that the enforced bacterial invasion during the first days of life might be conducive to the development of pylorospasm.

As an etiologic factor enlargement of the adrenals has also received attention (Pirie et al. 1919, Fanconi and Landolt 1949), this condition having been observed in infants with pyloric stenosis. However, according to another series (Tähkä 1951) no supernormal adrenals could be established in such cases, nor an enlarged fetal cortex.

There has been shown to be a significant excess of male infants in all the series published. 75—85 per cent of those affected are male infants (Salmi 1937, Pouyanne 1938, Ladd et al. 1947, Donovan 1946, Gripenberg et al. 1953). In about 50—60 per cent of all cases the disease has been found to occur in the first child of a family (Robertson 1940, Ladd et al. 1947), and in identical twins it has been encountered in both of them (Pouyanne 1938, Robertson 1940, Ladd et al. 1947).

With regard to the permanence of the tumor, it has been found in the follow-up examinations of children with hypertrophic pyloric stenosis who have been given different treatments, that in those treated conservatively or gastroenterostomized, the tumor remains permanent (Donovan 1946, Belding and Kernohan 1953).

On the other hand, in cases where pyloromyotomy has been performed, the tumor disappears in 7 weeks, according to one investigation (Donovan 1946). However, it has been established roentgenologically (Andresen 1940) that operated and non-operated cases have a narrow canalis egestorius with nearly equal frequency. According to this finding, pyloromyotomy cannot be considered to interrupt the course of the anatomic changes to any noteworthy degree.

Attention has also been given, in the study of the causes of hypertrophic pyloric stenosis, to the contribution of the autonomic nervous system. Infants affected with the disease have been followed into adulthood and it has been established (Schippers et al. 1936, Bendix and Necheles 1947) that they have a far higher tendency towards peptic ulceration and other gastrointestinal disturbances than others under similar conditions and that even their relatives display a higher than normal autonomic and neural lability. Salmi (1941) and Nieminen (1952) have likewise observed higher nervous lability in the follow-up examinations of children who have had hypertrophic pyloric stenosis than in the control material.

In histologic investigations changes have been observed in the nerve tissue of the pylorus. Herbst (1934) examined two stomachs of infants affected with pylorospasm (in a third case the stainings were not successful). The patients had been operated on (pyloromyotomy) but had died about 11 days later from purulent peritonitis. Herbst observed changes in Auerbach's plexus in the region of the pylorus and in the entire stomach. In the latter the changes were slighter. In the nuclei she established pycnosis as well as hypertrophy. In some of the nuclei a contraction and thickening of the nuclear membrane was observable, in others it was absent entirely or in part. The nucleoplasm was slightly granulated. It sometimes happened that instead of nucleoli only strongly stained chromatin granules of irregular shape were seen in the nucleus, surrounded by an annular vacuole. In the cytoplasm she frequently noticed a complete disappearance of the typical fibrillary structure characteristic of a normal cell. In some ganglion cells lumpy aggregations were visible in the cytoplasm, which she interpreted as residues of degenerated fibrils. Vacuoles were also observed abundantly in the cytoplasm. Furthermore, sometimes two ganglion cells were observed to be situated side by side and cytoplasmic fibrils were seen to link these cells as if they were in the process of

fusing together. Herbst also found binuclear cells. These phenomena she considered possibly to indicate a regenerative process.

In the nerve fibers Herbst observed the disappearance of the distinct fibrillar structure of the axis cylinders. Frequently, too, they lost their stainability. Moreover, disintegration and thickenings occurred in the fibers.

Herbst has furthermore noticed infiltrations of small cells in the ganglia in addition to the nerve fiber bundles. She interprets them either as pycnotic nuclei of ganglion cells or as motile connective tissue cells. Herbst employed Bielschowsky's staining method.

Niero (1947) has similarly used Bielschowsky's staining method in his investigations of the nervous tissue. He has particularly emphasized the fact that the ganglia in Auerbach's plexus are most numerous in the sphincter itself. Likewise, the atypical structur of the different kinds of tissue, the muscular, connective and nervous tissue, is at its most remarkable at this point. Of degenerative changes, he has observed vacuolization of the cytoplasm, nuclear disintegration of numerous nerve cells and the breaking off of nerve fibers. In the interstices of ganglion cells he has observed numerous satellite cells.

For the staining of ganglion cells Belding and Kernohan (1953), in their investigation of pyloric stenosis, have made use of cresyl violet, and for the staining of nerve fibers of Bielschowsky's method. According to their observations the myenteric plexus of the stomach is normal, but considerable changes have taken place in the pylorus. In numerous cells the nuclear membrane has disappeared. The normal location of the nuclear chromatin has undergone a change. The nucleolus has separated into two or three segments, or has disappeared entirely. The cytoplasm is weakly stained and lacks the fibrillar reticulum and the vacuoles characteristic of the cytoplasm of a normal ganglion cell. Frequently only cell contours are visible in the ganglion.

II. PROBLEMS

It is evident from the preceding that the occurrence of hypertrophic pyloric stenosis and its cause have been intensively studied. A great number of clinical observations have been made, on which various opinions are based. The nervous system of the pylorus, on the other hand, has received relatively little attention and the observations have been limited by the technic employed; moreover, they relate to rather scanty material. As has been mentioned in the foregoing, the intramural nervous system of infants suffering from hypertrophic pyloric stenosis has been investigated in detail by Herbst (1934), by Niero (1947) and by Belding and Kernohan (1953). These authors have employed Bielschowsky's method for the staining of nervous tissue, the last-mentioned also using cresyl violet. Their investigations have primarily thrown light upon the structure of the nerve fibers. Thus, for instance, Niero presents pictures of nerve fibers only -- not one single picture of nerve cells. At the same time, however, conclusions with regard to nerve cells have also been drawn. Since, however, Bielschowsky's method may cause artefacts, and moreover its reactions with the main constituents of the nerve cells, the nucleic acids and proteins, are not, as far as the author is aware, fully understood, it is probable that where this staining method has been employed, caution should be exercised in evaluating the cytochemical state of the nerve cells. Since, moreover, Herbst employed material from cases with peritonitis in addition to pyloric stenosis, peritonitis also being able to affect the histologic picture of the nerve cells, it was appropriate to study on a more exact basis the histologic changes in the intramural nerve tissue of the pylorus of infants affected with hypertrophic pyloric stenosis. The following questions arise, the answering of which will constitute the aim of this work:

1. Whether any histopathologic changes are observable in the myenteric plexus of the pylorus of infants suffering from hypertrophic pyloric stenosis.

2. What is the nature of these changes, if present.

3. Whether any changes in the histopathologic picture are observable during the course of the illness.

4. Whether it is possible to conclude, on the strength of the material available, whether the observed pathologic changes are rectified at recovery or whether they leave permanent traces.

5. Whether it is possible, from the observations, to draw conclusions with regard to the etiology of the changes.

6. Whether it is possible to explain the clinical symptoms and the recovery on the basis of the observations.

III. THE NEUROMUSCULAR ANATOMY AND PHYSIOLOGY OF THE PYLORUS

In order to obtain a clear conception of the nature of infantile hypertrophic pyloric stenosis, it is necessary to consider the neuromuscular anatomy and physiology of the pylorus in some detail.

A. MUSCULAR TISSUE

In its anatomic structure the pyloric ring is a typical sphincter with an opening as well as a closing mechanism (Horton 1928). Of the longitudinal muscle fibers, about 50 per cent traverse the circular muscle obliquely, attaching to the submucosa and constituting the opening muscle of the pylorus. Some of the longitudinal muscle fibers of the pars pylorica continue without interruption into the duodenum. In the closing circular muscle, on the other hand, a complete block is observable between the pars pylorica and the duodenum. This is effected by irregular connective tissue septa (v. Aufschnaiter 1894, Horton 1928).

The circular muscle system of the stomach has been observed to develop in the human embryo at the 23—41 mm stage (Lewis 1912, Welch 1921—22, Horton 1928), but the longitudinal muscle layer does not develop until the 41—75 mm stage (Lewis 1912, Welch 1921—22). However, segments in the longitudinal muscle layer are still absent at birth (Horton 1928), and they do not achieve full development until about one year subsequently (Welch 1921—22).

Belding and Kernohan (1953) have found that the longitudinal muscle fibers in the pyloric canal of infants suffering from hypertrophic pyloric stenosis continue from the pylorus into the duodenum in the same way as in normal infants and that longitudinal

muscle segments are absent from the pyloric canal in both instances. Moreover, according to these authors, a similar fibrous block exists, separating the circular muscle layers of the pars pylorica and the duodenum, as in normal infants. According to Niero (1947) in hypertrophic pyloric stenosis the outermost fibers (about one third) of the longitudinal muscle layer run from the pars pylorica to the duodenum; the bundles in the middle assume a vortical arrangement, and the innermost fibers traverse the circular layer obliquely towards the submucosa. According to the latter author the longitudinal muscle fibers take a similar course in the fetus in the fifth intrauterine month.

B. NERVOUS TISSUE

The nervous system of the pylorus is made up of the extrinsic and intrinsic nerves.

1. EXTRINSIC NERVES

The extrinsic nerves are composed of parasympathetic and sympathetic fibers entering the pylorus from the outside.

The parasympathetic nerves of the pylorus. The contribution of the vagus to the nerve supply of the stomach has been studied thoroughly and it has been found (McCrea 1924, Irwin 1931) that from the anterior and posterior vagus branches depart only up to the incisura angularis of the stomach, but not below this level. The vagus nerves of the pyloric region, again, originate from the hepatic branch of the anterior vagus.

The sympathetic nerves of the pylorus. The sympathetic fibers have been found (McCrea 1924) to enter the stomach from the celiac plexus, and they provide all parts of the ventricle with nerves.

2. INTRINSIC NERVES

The intrinsic system is composed of two distinctly observable plexuses: the *myenteric* (Auersbach's) and the *submucous* (Meissner's) plexus. Lawrentjew (1931) divides the intrinsic nerves into four systems: the subserous, the myenteric, the deep muscular, and the submucous system. According to Schabadasch (1930) there is, moreover, the plexus of Henle between the deep muscular and the

submucous system. Stöhr (1949, 1951) divides the system in question into six plexuses.

The myenteric plexus (Auerbach's plexus) is located between the outer longitudinal muscle layer and the inner circular muscle layer. It extends without interruption from the pars pylorica to the duodenum (Horton 1928, Irwin 1931). The location of the nerve plexuses in the pyloric canal is shown in fig. 1.

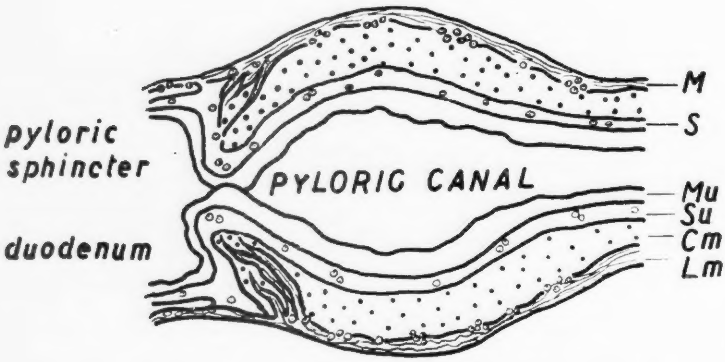


Fig. 1. — Schematic diagram of the pyloric region of a normal infant. MU = mucosa; SU = submucosa; CM = circular muscle layer; LM = longitudinal muscle layer; M = myenteric plexus (Auerbach's plexus); S = submucous plexus (Meissner's plexus).

The number of ganglion cells is highest in the pyloric region as compared with the rest of the digestive tract. In comparison with the middle third of the esophagus, for instance, the number of nerve cells is about tenfold (Irwin 1931). The nerve elements of the myenteric plexus are accompanied by satellite cells, which are smaller than the ganglion cells. They are of elongated form and have a scarcely observable cytoplasm. The nuclei are oval as a rule and the nucleolus is diminutive. There are still other cells in the myenteric plexus, which are similar in appearance to the nerve cells but of smaller size. They have a minute, but observable cytoplasm. According to Stöhr (1949, 1951) they are immature nerve cells which may develop into nerve cells in case of need.

3. THE RELATION OF THE PARASYMPATHETIC AND SYMPATHETIC FIBERS TO THE INTRINSIC SYSTEM

In her investigations, Hill (1927) observed that the vagus fibers formed a synaptic connection in the myenteric plexus of the stomach and the intestinal wall with associative, or Dogiel I type, cells. These, in turn, are synaptically connected with motor, or Dogiel II type, cells. The latter extend nerve fibers directly to the individual muscle fibers in the longitudinal and circular muscle layers. Hill was not able to find any synaptic connection in the myenteric plexus to the postganglionic fibers. These fibers travelled direct to the submucosa, where they terminated in the submucous glands and in the mucosa.

Van Campenhout (1930), who has studied chick embryos, has arrived at the conclusion that the enteric intrinsic system is of sympathetic origin. Müller (1921), Müller and Ingvar (1921, 1923) and Lawrentjew (1929), however, believe that the myenteric plexus has a twofold origin, parasympathetic as well as sympathetic.

C. PHYSIOLOGY

In their investigations on the motility of the stomach, McCrea et al. (1924) divided this organ into two functional parts, namely, the *reservoir*, including the fundus and the proximal part of the corpus ventriculi, and the *motor segment*, to which belong the remaining parts of the corpus ventriculi and the pars pylorica. Their line of demarcation is the *lower segment loop* in the vicinity of the incisura angularis observed by Forssell (1912). Cathcart (1911) named this loop the *prepyloric sphincter*. The pyloric ring and the pyloric antrum thus constitute a functional unit, upon which the extrinsic nerves have the same stimulating effect (Van Braam-Houckgeest 1872, 1874, Thomas and Wheelon 1922, McCrea et al. 1924). According to the results of their experiments, the pylorus is supplied with both vagus and splanchnic fibers, although they are not directly antagonistic to each other (McCrea and McSwiney 1926, 1928, Nolf 1925), both containing motor as well as inhibitory fibers. However, the inhibitory fibers are more abundant in the splanchnicus than in the vagus. According to certain experiments, upon resection of the splanchnicus vagal stimulation resulted in vigorous motility of the stomach (Van Braam-Houckgeest 1874, McCrea et al. 1925, Barron 1939). Upon vagotomy, on the other hand, the

peristaltic waves completely diappeared in the human stomach after ten days (Hightower 1953). The way in which the pylorus reacts to vagus and splanchnicus stimulation is considered to be a result of the initial state of tension of the pylorus, its tonus (McCrea and McSwiney 1926).

Gastric movement is composed of two separate phases according to some investigators (McCrea et al. 1924):

the peristaltic wave, involving the corpus ventriculi and its preantral part, and

the contraction of the musculature of the pyloric antrum as a whole.

It thus includes simultaneously the peristalsis of the corpus ventriculi and the systole and diastole of the pyloric antrum.

The opening and closing of the pylorus has been studied by Klee (1912), who used the fluoroscope. He has observed the movements of the stomach elicited by vagal stimulation while it contained a barium meal. According to his observations, the pyloric ring relaxed shortly before a wave of contraction reached the pylorus, if it carried an adequate mass of the stomach contents before it. The pylorus did not open at the approach of a peristaltic wave when a considerable mass of the stomach contents had passed into the duodenum and still remained there. The pyloric action thus appears to be controlled by a reflex mechanism involving both the stomach and the duodenum.

In their studies of the intestinal movements, Bayliss and Starling (1901) have arrived at the conclusion that these movements are controlled by a local, intrinsic nervous system. They have also established that the peristaltic contractions in the isolated intestine are elicited by the local nerve plexus.

According to the present view, the action of the pyloric muscle is regulated by reflexes partly originating in the stomach and partly in the duodenum (Thomas 1931). The reflexes are transmitted by the myenteric plexus and the vagus. Consequently the myenteric plexus plays a central role in the functioning of the pylorus and has for this reason been chosen as the subject of the present histologic investigation. From this angle an attempt will be made to detect possible nerve lesions which might constitute an etiologic factor contributing to the occurrence of hypertrophic pyloric stenosis, in accordance with the neurogenic conception advanced by Räihä (1948).

IV. THE CHEMICAL CHANGES INDUCED IN ADEQUATE NERVE CELLS BY CERTAIN STIMULI

Because it has been shown experimentally that numerous stimuli may produce chemical changes in adequate nerve cells, some experiments and their results will be reviewed here in order to obtain a basis of comparison for the histochemical changes observed in the present work.

1. CYTOCHEMISTRY OF THE NERVE CELL

The cell matter of a nerve cell undergoes a continuous process of change associated with the function of the cell. In the metabolism of the neurone the nucleus plays a dominant role. Proteins and nucleic acids are of central importance among the cell substances; the production of proteins takes place with the aid of the nucleic acids.

The nerve cells, which represent the largest somatic cell type of the mammals, differ from other cells during their development with regard to volume and the formation of the axon. When a unipolar neuroblast develops into a multipolar one, its protein increases more than five-fold in quantity. In most of the other somatic cells growth comes to a standstill at this point, and the protein-producing system of the cell diminishes. The nerve cell, on the other hand, goes through another growth period. When the multipolar neuroblast develops into a full-grown nerve cell, the protein of its cytoplasm increases about 2000-fold, and the growth of the axon requires a further enormous amount of proteins (Hydén 1943a). During this stage of growth the protein-producing system develops greatly and displays signs of enhanced activity: a large nucleolus rich in nucleic acids is visible and ribonucleotides are abundantly present in the cytoplasm.

Modern technics have provided cytochemical research with the means to investigate the composition and metabolism of the nerve cell. During the last decade, Hydén and his collaborators have studied these questions in numerous valuable investigations, employing the ultraviolet absorption and roentgen-microradiographic technics in the analysis of the nerve cells and the localization of their constituents. Since these methods of investigation form the basis on which a comparison of the staining and of the histochemical changes is possible, it is considered appropriate to include here a description of their application to the study of nerve cells.

The *ultraviolet absorption method* has been elaborated by Caspersson (1936, 1940). It is based on the fact that nucleic acids have a strong absorption at 2600 Å on account of the purine and pyrimidine bases. The proteins have their absorption maximum at about 2800 Å, owing to their content of tyrosine, tryptophane and phenylalanine. An absorption spectrum in ultraviolet light between 2400—3200 Å of the part of the cell under investigation is first photographed, whereupon the spectrum obtained is analyzed. The quantity of nucleic acid per unit volume of the cell can be computed from the extinction values, assuming the specific weight to be 1. This determination can be carried out with respect to parts of the cell down to 1 μ. The use of Feulgen's test makes it possible to decide whether the nucleic acid is of the ribose or the ribodesose type. The number of free amino groups can be considered a measure of the hexone base content. If the section is stained with an acid stain and the maximum intensity of the staining determined in the part of the cell under investigation, it is possible to calculate the number of bound dye groups, which can be considered an approximate measure of the basic groups of the cell protein.

By this method much light has been shed on the composition and function of the nerve cell. Nevertheless it has its own limitations. The determinations are rendered inaccurate by the inhomogeneity of the protein composition of the cell, which also restricts its applicability (Glick et al. 1951). The protein content can be computed from the amino acid extinction values at 2800 Å as an approximation only, because a comparison has to be made with a protein standard containing the various acids in fixed proportions. When the absorption is calculated at 2600 Å, errors are caused by scattered

light and by the losses of light occurring between particles differing in refractive index. However, this method yields satisfactory semi-quantitative values for the nucleic acid contents if it is employed in optically corrected conditions.

With the aid of ultraviolet spectrography it is thus possible to locate and determine only one of the cytochemically important groups of substances, namely, the nucleic acids.

The *roentgen-microradiographic* technic affords to cell research more extensive possibilities. Brattgård and Hydén (1952) have recently devised a new method for the determination of lipins, pentose nucleoproteins and other proteins of the cell. The analysis is carried out roentgen-microradiographically at 8—10 Å from an untreated section. The different fractions are obtained from the weight differences, after extraction with solvent and enzymatic decomposition. The quantity of substance is obtained with an accuracy of 10^{-9} mg/μ^3. The area analyzed is 4 μ^2 and the procedure is repeated to cover the whole cell. The method is based upon the principle, developed by Engström and Lindström (1949, 1950), of determining the mass of a part of the cell with the aid of roentgen-microradiography. The mass of biologic matter is proportional to the absorption of radiation at 8—10 Å (taking into account: H, C, N, and O).

In their application of this method to the investigation of nerve cells, Brattgård and Hydén have determined the sources of error and made several important technical improvements to the method. As sources of error there are mentioned: the margin effect and the so-called Kostinsky effect, the change in volume and thus in mass of the section on extraction with chloroform and on disintegration with enzyme, the escape on extraction and enzymatic disintegration of substances other than those intended, the residual dry matter after enzymatic disintegration, etc. Several of these sources of error can be eliminated, or their influence reduced. Taking into account the photographic process and the determination of the thickness of the section, the aggregate error in the roentgenographic determination is, according to the said authors, 2—3 per cent.

Study of the nerve cells by means of ultraviolet spectrography reveals a large nucleolus containing ribonucleotides and proteins of strongly alkaline character. This substance of the nucleolus occurs as small particles in an extensive network. These particles

also contain varying amounts of ribodesose nucleic acids. The particles are considered to be chromatin associated with the nucleolus (Hamberger and Hydén 1945). In the large ganglion cells of most mammals an area is observed close to the nucleolus, which has a stronger absorption of ultraviolet light than the surrounding region. This area also stains deeply with acid dyes. It gives a characteristic absorption spectrum which reveals its content of pentose nucleic acids, both of ribose and ribodesose type, and of proteins of remarkably basic nature. The protein concentration is about three times that of the surrounding nuclear substance. This protein resembles in its composition a chromosome constituent of various dipterous insects, and it is more abundant in male than in female animals (Hydén 1943a). For this reason it can be compared with the Y chromosome. The region in question is called the heterochromatic centre and it is part of the chromatin attached to the nucleolus. The heterochromatic centre has been investigated in the case of the rabbit. It has been found to react strongly to stimulation (Hamberger and Hydén 1949 a, 1949 b) and to virus infection (Hydén 1947), during which the heterochromatic substance may increase.

In the cytoplasm, ribonucleotide accumulation and an abundance of protein masses are observed. The nucleotides of the cytoplasm are of the ribose type. The distribution of the nucleotides seems to be in accordance with the distribution of Nissl bodies (Hydén 1943a). Thus the bulk of the Nissl basophilous substance is composed of ribonucleotides of high acidity, and proteins.

The cytoplasmic nucleotides in the nerve cell have a functional character and a certain relation to the formation of proteins which characterizes increasing cell activity. Hydén's school has been able to demonstrate this experimentally in numerous states of stimulation, which will be discussed later. In these tests a decrease of the nucleotides and proteins in the cytoplasm has been observed, which manifests itself in the histologic picture as tigrolysis.

The proliferation and growth of the cell are both attended by protein production. Caspersson and Schultz (1938) have shown that this process requires the presence of nucleic acids and that the function is confined to the cell nucleus. The production of gene protein takes place with the assistance of the ribodesose nucleotides, whereas the proteins of the cell body are formed under the influence

of the ribose nucleotides. These substances are responsible for the synthesis of the protein continuously consumed in the cell body. One part of the nuclear chromatin, the heterochromatin, and the nucleolus play a central part in this production of cytoplasmic protein. These two constituents of the nucleus are characterized by a high content of proteins rich in hexone bases. The basic proteins travel from the heterochromatic chromocentre and from the nucleolus towards the nuclear membrane, where the cytoplasmic protein is formed with the assistance of the ribose nucleotides. *The degree of development of the system nucleolus-nuclear membrane can thus be considered an indication of the intensity of the protein metabolism.*

Hydén (1943b) has been able to prove a relation between the system nucleolus-nuclear membrane and the production of cytoplasmic protein. He has investigated the different phases of protein formation under physiologic conditions in the spinal ganglion cells of a certain teleost (*Lophius piscatorius*). Fig. 2. shows a spinal ganglion cell of *Lophius piscatorius.* Basic protein flows from the large nucleolus towards the puckered area of the nuclear membrane. Simultaneously ribonucleotides are accumulated abundantly close to the nuclear membrane in the cytoplasm. In this region cytoplasmic proteins are also formed. The phenomenon can be considered an indication of the central role of the nucleus and particularly of the nucleolus with regard to the formation of pentose nucleoproteins

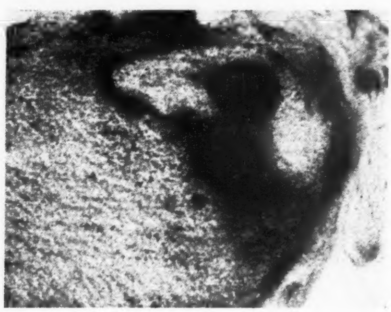


Fig. 2. — The giant nucleolus is directly connected with the area rich in nucleotides and protein between the nucleolus and the nuclear membrane and with the nucleotides of the nuclear membrane. Ultraviolet photograph. Magnification × 1100. (Hydén 1943b.)

Fig. 3a. — A group of nerve cells from the cochlear ganglion of acoustically non-stimulated guinea-pigs. The cells are unstained, merely fixed in Carnoy's solution and photographed near the absorbtion maximum of the nucleic acids at 2570 Å. In the nuclei, which absorb moderately, the nucleoli, rich in nucleic acid, stand out distinctly owing to their intense absorbtion. The cytoplasms as a whole absorb intensely, especially so in certain parts. The latter contain ribose nucleotides as well as proteins and correspond in the cytological picture to Nissl substance. Magnification × 2000. (Hamberger and Hydén 1945.)

Fig. 3b. — Nerve cells from the cochlear ganglion of guinea-pigs, photographed at 2570 Å about 48 hours after acoustic stimulation with 6000 cycles and 80 dcb for three hours. The cells as a whole absorb weakly. Distinctive of these cells are the rings of intensely absorbing substance surrounding the nuclei, the nuclear-membrane nucleotides (NMRN), which are typical of nerve cells with a stimulated protein-forming system. Magnification × 2000. (Hamberger and Hydén 1945.)

Fig. 3c. — Group of nerve cells from the cochlear ganglion of guinea-pigs killed 2 weeks after acoustic stimulation with 6000 cycles and 80 dcb for three hours. The cells were photographed at the absorbtion maximum of the nucleic acids. They are marked by a very slight absorbtion. The cytoplasms are seen on the photograph to be homogeneous. Only the nucleoli have retained their intense absorptive power. Magnification × 2000. (Hamberger and Hydén 1945.)

Fig. 3d. — A group of nerve cells from the cochlear ganglion of guinea-pigs killed at the beginning of the third week after acoustic stimulation with 6000 cycles and 80 dcb for three hours. A distinctive feature of this stage is the appearance of intensely absorbing rings round the nuclei, the nuclear-membrane nucleotides (NMRN), which are typical of this stage of incipient regeneration. Magnification × 2000. (Hamberger and Hydén 1945.)

Fig. 3e. — A group of nerve cells from the cochlear ganglion of guinea-pigs, examined at the end of the third week after acoustic stimulation with 6000 cycles and 80 dcb for three hours. The cytoplasms as a whole absorb rather intensely at 2570 Å. Magnification × 2000. (Hamberger and Hydén 1945.)

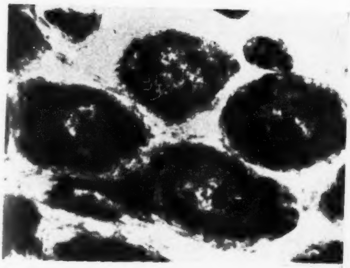


Fig. 3 a

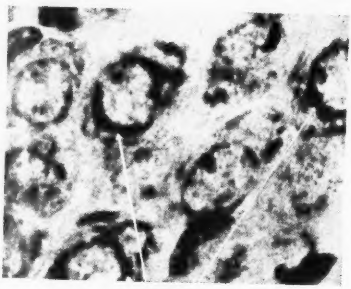


Fig. 3 b

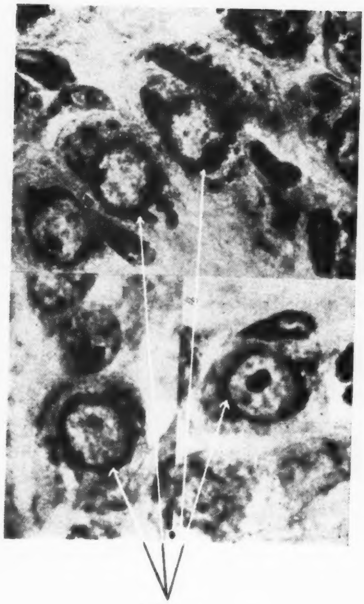


Fig. 3 d

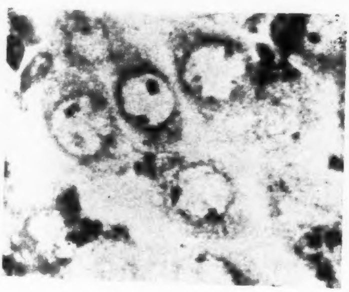


Fig. 3 c

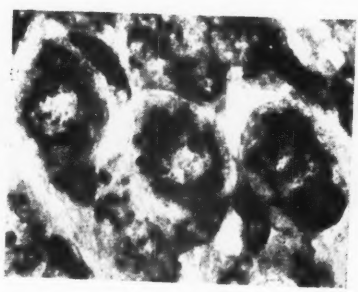


Fig. 3 e

in the cytoplasm. The possibility is not excluded that the cytoplasm may further possess a system of its own for the formation of nucleic acids.

2. ACOUSTIC STIMULUS

Hamberger and Hydén (1945) have studied the chemical changes caused by acoustic stimulation of the nerve cells of the cochlear ganglion, employing various cytochemical methods. The stimulation was produced by means of a physiologic acoustic stimulus (audiometer) and by means of trauma (shooting). The methods of investigation were ultraviolet absorption spectrography and histologic staining. The control series consisted of guinea-pigs which had not previously been subjected to acoustic trauma.

Investigation of the normal series with the ultraviolet absorption method showed that the nucleolus had a strong absorption at the absorption maximum of the nucleotides. The other portions of the nucleus had a relatively low absorption, whereas the cytoplasm absorbed intensely (fig. 3a). The protein content of the cytoplasm was 30—40 per cent according to the said authors and the nucleotide content 2—4 per cent.

Physiologic acoustic stimulation

In the physiologic acoustic stimulation the frequency and intensity of the sound were varied. The duration of the stimulation was 3 hours. The changes in the nerve cells were observed for 3 weeks. The first changes were noticed 24 hours after termination of the stimulation. In the cytoplasm small regions with lower absorption than the other parts of the cytoplasm were observed. After 48 hours there was a very intensely absorbing area in the cytoplasm close to the nuclear membrane (fig. 3b), where ribonucleotides and proteins were formed abundantly. One week after stimulation, the major part of the cytoplasm absorbed rather weakly, and after two weeks the ganglion cells contained only a small amount of nucleic acids (fig. 3 c). Tigrolysis was established with the aid of an acid dye. This tallies with the observed low nucleic acid content. At the beginning of the third week most of the cells displayed signs of incipient regeneration. The nuclei were surrounded by rings showing an intensive absorption at the absorption maximum of the nucleic

acids (fig 3d). The absorption spectrum revealed that this region contained an abundance of nuclear membrane nucleotides. This is an indication of increased activity of the protein-producing system in the nerve cell. At the end of the third week the nerve cells display an even stronger regeneration, approaching restitution (fig. 3e). It was concluded from the absorption spectrum that the cytoplasm is rich in nucleotides and proteins. Thus during the third week the original concentration of nucleic acids and proteins had been restored in the ganglion cells.

The course of these changes has been plotted graphically in fig. 4. It shows that the first changes comprise a decrease of the nucleotides and proteins in the cytoplasm. The nucleotides of the nuclear membrane are at the same time abundant, which is an

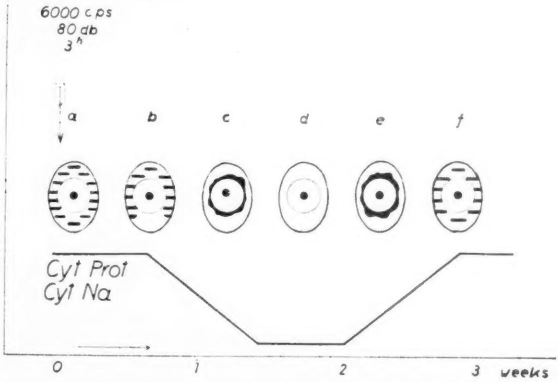


Fig. 4. — Diagram illustrating the extensive protein-rebuilding processes in connection with acoustic stimulation, in the present case with 6000 cycles and an intensity of 80 dcb for three hours. The thick, unbroken curve indicates the changes in the nucleic acid and protein concentration in the cytoplasm (Cyt Prot and Cyt Na). The abscissa shows the lapse of time in weeks after the stimulation (marked by an arrow). The horizontal dashes and dark areas in the cells indicate ribose nucleotides. After the stimulation with sound, the first changes occur in the cytoplasm, in which the nucleotide and protein concentration diminishes within an often sector-shaped area (b). In the course of the first week the concentration of these substances gradually diminishes. Round the nuclei, however, nuclear-membrane nucleotides appear in large concentrations, indicating intensive excitation of the cellular protein-forming system (c). In the second week the effect of the acoustic stimulation on the cells reaches its maximum. Only scanty amounts of proteins can then be observed in the cells (d). In the course of the third week a successive restitution of the cells takes place. Its commencement is indicated by the appearance of nuclear-membrane nucleotides (e). By the end of this week the original nucleotide and protein concentrations have been restored (f). (Hamberger and Hydén 1945.)

indication of increased formation of protein. During the first week after acoustic stimulation the protein content falls and during the second week it amounts to only 2—10 per cent. Nucleotides can no longer be observed at this stage. During this time the function of the protein-producing system is inhibited. In the third week the nucleotides of the nuclear membrane again appear in large quantities, this being a sign of intense formation of cell protein, and after three weeks the concentration of proteins and nucleotides has regained its former level.

Acoustic trauma

The experiments were extended to include acoustic trauma in order to determine whether in this case the chemical changes possibly differ from those effected by acoustic stimulation within physiologic limits. Trauma was achieved by 12 pistol shots daily during 6 days, at a distance of 30—40 cm from the experimental animal. Even during the first week after termination of the experiment the changes were extensive. The cells were throughout characterized by a low power of absorption at the absorption maximum of the nucleotides. The nucleoli displayed a comparatively low absorption. It was evident from the absorption curve that the concentration of nucleotides and proteins was low, and this state could be observed as late as 8 weeks after termination of the acoustic trauma. No increase was observable in the amount of nucleic acids and proteins during this time, but the frequency of occurrence of nuclear membrane nucleotides was considerably higher than in the first stage immediately upon termination of the experiment. This phenomenon is an indication of incipient restitution.

3. ROTATION STIMULUS

Hamberger and Hydén (1949b) have studied the formation of nucleoproteins in the nerve cells of the vestibular ganglion and of Deiters' nucleus, using rotation as a stimulus. The investigation includes normal conditions (control), increased activity achieved by means of rotation and activity resulting in exhaustion.

The method of investigation was ultraviolet absorption spectrography, assisted by histologic staining and disintegration with ribonuclease.

Changes in the vestibular ganglion

The nerve cells of the vestibular ganglia of the control animals have a strongly developed nucleolar apparatus, and in the cytoplasm the ribonucleic acids and proteins occur in aggregations. On stimulation of the animals by rotation for 8 minutes twice during one day (intensive stimulation) no changes in the duration of the postrotatory nystagmus were observed; thus the function of the vestibular apparatus was still normal. The stimulation caused a considerable increase in the total concentration of nucleoproteins in the nerve cells.

On increase of the stimulus so that rotation took place for 8 minutes every day during 6 days, a reduction of the duration of the postrotatory nystagmus could be observed after the third day. This can be considered a sign of physiologic fatigue. However, conditions were not pathologic, as the animals were able to perform complicated movements and the vestibular defence reflexes were normal. In the ultraviolet absorption picture this state is seen as a decrease in the total amount of ribonucleic acids and proteins in the cytoplasm of the vestibular ganglion cells.

On determination of the shortest time of stimulation sufficient to produce cytochemically observable changes in the vestibular ganglia, this was found to be 2 minutes. The increase of the amount of nucleoproteins could be established 30 minutes after termination of the test. The initial composition was restored in 24 hours. With the intense stimulation described the original chemical composition was restored after about 48 hours.

On the basis of the experiments reviewed above, the authors concerned have concluded that an increase and decrease of the ribonucleic acids and proteins takes place in the nerve cells of the vestibular ganglion. These changes are correlated with nerve function and the demands made by that function.

Changes in Deiters' nucleus

An investigation was also made of the nerve cells of Deiters' nucleus, which constitute the second neurone in the vestibular tract, in conditions similar to those of the nerve cells of the vestibular ganglion. The results show that the nerve cells of Deiters' nucleus react upon slight stimulation, in that the substance of their

chromocentre region increases. These regions constitute part of the system primarily responsible for the formation of the nucleoproteins in the cytoplasm. The changes in question indicate a vigorous activity of this system. Stronger stimulation causes an increase of the nucleoproteins in the entire nerve cell. These changes are to be regarded as manifestations of increased nervous activity. By means of a rotation stimulus sufficient to produce fatigue a quantitative decrease of the nucleic acids and proteins in the nerve cells of Deiters' nucleus is achieved.

4. MOTOR STIMULUS

Muscular exertion causes extensive chemical changes in the rhabdomyotic cells concerned (Hydén 1943a, 1944). This has been observed in experiments with guinea-pigs. In these animals the motor cells of the anterior horn are characterized by a high content of nucleic acids and protein. When guinea-pigs were made to run to exhaustion on a tread mill, ultraviolet spectrography revealed in the nuclei of the cells concerned only a sparse amount of basic proteins and in the cytoplasm only a small amount of proteins and hardly any nucleotides. The difference in the content of these substances between tired and thoroughly rested animals was 3 to 5-fold. In the nucleolus the amount of basic proteins was considerably increased in tired animals, which is an indication of intensive activity on the part of the protein-producing apparatus. Nissl's staining gave a typical picture of tigrolysis.

It has also been possible to confirm this result with the aid of roentgen-microradiographic technic (Hydén 1952). In guinea-pigs which had run to exhaustion the amount of pentose nucleoproteins was found to be only one fifth of that of the control animals. The protein fraction had remained nearly unchanged. The changes can be observed as early as 1 hour after termination of the test, and the initial concentration is restored after about 50 hours.

5. ELECTRIC STIMULUS

If a weak electric current (1—2mA) is allowed to act upon the spinal ganglion cells for 5 minutes, the protein-producing system is stimulated rather strongly and the average concentration of the cytoplasmic nucleotides increases from 1 per cent to 6 per cent (Hydén 1943a, 1944). The concentration of the proteins increases

in the cytoplasm during stimulation to four times its initial value. If the stimulus is continued for 10 minutes, the amount of nucleotides and proteins in the cytoplasm falls abruptly, but the nucleolar apparatus is very active. The histologic picture shows tigrolysis.

6. CHEMICAL STIMULUS

Insulin shock

The influence of insulin shock upon the cells of the anterior horn of the spinal cord has been investigated with rats by inducing convulsions in them for a few hours with the aid of insulin (Hydén 1944). In the cells of the anterior horn Nissl's stain reveals tigrolysis, and the results obtained by means of cytochemical methods show that the nucleotides and proteins are greatly decreased in the cytoplasm. In the protein-producing system signs of intense activity are observable. The amount of basic proteins, in particular, is increased in the nucleolar apparatus and in the nucleus. After one week the protein concentration in the cytoplasm was restored.

Streptomycin

It is well-known that therapeutic doses of streptomycin cause symptoms in the vestibular nervous system which may interrupt its function. Floberg et al. (1949) have directed their investigations towards an elucidation of this phenomenon. As experimental animals they used guinea-pigs of 300—500 g weight, which were given 25 mg of streptomycin three times a day intramuscularly during 3—28 days. Vestibular function was checked by means of rotation. The duration of the postrotatory nystagmus decreased continuously and the nystagmus ceased altogether after a time of varying length.

In guinea-pigs which had been given altogether 750 mg of streptomycin during 10 days, postrotatory nystagmus could no longer be observed. In the cells of the vestibular ganglion great cytochemical changes were visible. Only a small amount of proteins remained, and nucleic acids were no longer observable. The greatest quantitative changes were noted in the vestibular ganglia, but there were also considerable changes in Deiters' nucleus. Streptomycin thus causes profound chemical changes in the vestibular apparatus both in its peripheral and in its central parts.

The influence of streptomycin seems to be selectively limited to the neurones of the vestibular tract. Its effect is obviously that

of inhibition of the intracellular reproduction of nucleic acids. According to Massart (1948), streptomycin forms an adsorption complex with the ribonucleoproteins, and this complex is resistant to the influence of ribonuclease.

7. SEVERING OF THE AXON

Retrograde nerve cell degeneration has been studied in the ganglion cells of the hypoglossal nucleus and the nodose ganglion and in the spinal ganglion cells of the ischiadic nerve (Hydén 1943a). When the axons are cut off, a disappearance of proteins and nucleotides is seen in the nerve cells concerned during the regression process. In the histologic picture this is evident as tigrolysis. About two weeks after severance of the axon these changes have reached their maximum, and after one month signs of the formation of proteins reappear in the cells. When the activity in the neurone is restored, the nerve cell regains its initial nucleotide and protein concentration.

8. SPASTIC PARALYSIS

Hochberg and Hydén (1949) have investigated the nucleotide and protein metabolism of motor nerve cells in spastic paralysis produced by sufficiently prolonged (15 and 25 minutes) clamping of the rabbit's abdominal aorta. The animals were killed at varying times after termination of the clamping. After 15 minutes' clamping the paralytic symptoms appeared 2—12 hours after termination of the test and persisted for from 15 minutes to 8 hours. In mild spastic paralysis scanty absorption was observed in the nucleolus and in the cytoplasm, and the histologic picture showed tigrolysis. Minimum absorption obtains 15 minutes after termination of the clamping. After about 2 hours the nucleoprotein content had regained its normal concentration, and after 8 hours the amount of nucleoproteins exceeded that in the control cells.

When the abdominal aorta was clamped for 25 minutes, irreversible changes were observable in the motor nerve cells. Eight hours after the test a considerable decrease was noticeable in the nucleoproteins both in the cytoplasm and in the nucleolar apparatus. Six days and thirty days after the test there still remained structural residues of the nerve cells, but the majority had been phagocytized. Nucleic acids and proteins could no longer be established in the

nucleolar apparatus or in the cytoplasm. This nucleolar change is a serious prognostic sign in assessing the vitality of nerve cells. A cell devoid of an active protein-producing system is moribund and will gradually be phagocytized.

9. THE SIGNIFICANCE OF ADEQUATE STIMULATION IN THE DEVELOPMENT OF THE NEURONE

The time at which a stimulus impinges on the nerve cell has a fundamental influence upon the chemical composition of the neurone and at the same time upon its function. Brattgård (1952) has studied this question with the aid of the roentgen-spectrographic method. His object of investigation was the retina of the rabbit. The rabbits had been kept in complete darkness for 10 weeks following their birth. As control animals, rabbits were used which had lived for the first ten weeks after their birth in normal daylight.

The cellular substance of the animals born and subsequently kept in darkness amounted only to about one sixth of that of the control animals and no pentose nucleoproteins whatsoever could be established in their cells. The microradiograms gave the impression that the cells were mere empty shells. When the rabbits which had lived in darkness for ten weeks were exposed to daylight, pentose nucleoproteins appeared in their cells. The concentration seems to depend on the time after birth at which the light stimulus meets the nerve cell. After three weeks of living in the daylight the amount of pentose nucleoproteins in the experimental animals only equalled that in the control animals after 11 days.

This result is in accordance with practical experience. Senden (1932) states that human beings who have been blind since birth owing to cataract are only able to discern the shape of objects after long practice and that actual sight is never possible to them. Riesen (1947) obtained similar results in his experiments with chimpanzees which had spent the first 16 months of their life in darkness. Only after prolonged practice was a certain degree of distinctive sight achieved.

Consequently stimulation during the first period of life is of decisive importance to the functioning of the optic tract, both in its peripheral part and in the complicated functioning of the cerebral centre.

V. MATERIAL AND METHODS

A. MATERIAL

The material was obtained from the Children's Hospital of the University of Helsinki, the investigations having been carried out at the Institute of Forensic Medicine and the Department of Pathology of the University of Helsinki as well as at the Department of Pathology of the University of Copenhagen.

The material consists of a pyloric stenosis series and a control series. The former comprised 56 cases and the latter 18. The material contains both autopsy and biopsy findings. In the autopsy material the sections were taken from four points of the pyloric canal, namely, from the ventral and dorsal walls, and from the walls of the lesser and the greater curvature. The abbreviated notations P.ventr., P.dors., P.c.mi. and P.c.ma. refer to these locations. The biopsy specimens were taken from the ventral wall of the pylorus from the mucous to the serous membrane. Autopsy was performed as soon after death as possible (2—48 hours), as a rule before 24 hours had elapsed, but frequently only two hours after death. The fixing fluid was often introduced into the stomach with a tube, or injected intraperitoneally, immediately after death.

Upon fixation, the sample specimens were transferred to paraffin by way of xylene. The sections have a thickness of 5 μ.

B. METHODS

1. FIXING

Of the numerous possible fixing methods, formalin fixation and absolute alcohol — glacial acetic acid fixation were chosen. *Formalin fixation* was used in the majority of cases. This was considered expedient (Pearse 1954), since this method is known to preserve

the shape well, and the specimens may be kept for a considerable time in the fluid without loss of stainability. The influence of formalin fixation upon the tissue was also considered favourable with regard to gallocyanin-chrome alum staining, which was the most generally used staining method, because the pH of the formalin falls rapidly during fixation and remains approximately constant for a long time. Bendien and Gans (1927) have found that on fixation of cerebral material with formalin the acidity of the formalin rapidly decreased to pH 4.7 and then remained constant even for several years. This is probably due to methylenization of the amino groups of the proteins (Blum 1896/1897, Schwarz 1901) through the effect of formalin, the new compounds being more acid in character than the original constituents. These compounds probably contribute to the acidity of the formalin fixing fluid. Fixation in formalin thus takes place in a suitable environment from the standpoint of colloid chemistry, the various chemical changes in the proteins being nearly at a minimum. In so far as changes occur in the charges on the protein molecules, they are reversed again in the staining solution in accordance with the pH of this solution.

In view of the above-mentioned facts neutralization of the formalin prior to fixation was not considered necessary or desirable. Therefore, the formalin used was of the commercially available quality diluted with water (aq. dest.) to a concentration of 10 per cent with regard to formaldehyde.

Fixation was also carried out with *abs. alcohol-glacial acetic acid* (95 ml abs.alc. + 5 ml glacial acetic acid, Romeis 1943) the intention being to determine the part played by different fixatives in the picture obtained with the gallocyanin staining method employed in this work. However, no appreciable effect could be observed.

In one case *formalin—formic acid—alcohol fixation* was employed (5 ml formalin of 35 per cent by wt., 2—5 ml formic acid and 90 ml 80 per cent alcohol, Romeis 1943).

2. STAINING

a. For the staining of nerve cells, *Einarson's gallocyanin—chrome alum staining* (1932) was used. According to Einarson and Bentsen (1939) this method is to be regarded as considerably superior to

other stains where the selective demonstration of nerve cells is concerned. It permits an evaluation of reversible changes occurring in the shape of the nerve cells. Nissl bodies, the chromatin of the nucleus and the nucleolus, and the nuclei of the satellite cells are all stained. The nuclear membrane is likewise well stained and is made distinctly visible. The remaining tissue is stained less deeply, the depth of the stain depending on the hydrogen ion concentration of the staining solution. Lowering of the pH of the staining solution increases the specific staining of the nuclear chromatin and of Nissl substance. If the pH is increased, even the remaining tissue begins to take on a stain in addition to these constituents of the cell. A picture of the desired quality is thus obtained by regulating the pH of the staining solution within certain limits.

Gallocyanin and chrome alum combine to form a complex of basic character. This complex attaches selectively to the fixed, basophilous cell structure (Einarson 1951). The dye satisfies the affinity of the cell, and when the stain has achieved a certain intensity it does not increase further even if the staining time is prolonged. The stain is, moreover, very stable and is not affected by alcohol or xylene in the dehydration and fixing phases. The intensity of the stain is thus only dependent on the colour-binding capacity of the cell at the time of fixation, i.e., the electrostatic condition of the object stained.

Preparation of the staining solution and staining were carried out essentially in accordance with Einarson's (Romeis 1943) directions. For the preparation of the staining solution, 0.15 g gallocyanin (Hollborn & Söhne) was added to 100 ml of 5 per cent chrome alum solution ($CrK(SO_4)_2 \cdot 12H_2O$, pure, ironfree, Stuers) and the mixture was boiled for 5 minutes with simultaneous agitation. The solution was allowed to cool thoroughly and passed through filter paper. The filtrate, reduced in amount on account of evaporation, was restored to its original volume by washing the filter with distilled water. The staining solution obtained in this way had a pH of about 1.7.

The staining was performed in staining solutions of pH 1.7, 2.5 and 3.4. The regulation of the hydrogen ion concentration of the staining solution was achieved with the aid of 1 N NaOH or sodium carbonate-borax buffer solution.

α. 1 N NaOH was added in the proportion of 10 ml/100 ml of staining solution. Immediately upon addition the staining solution had pH 4.7, which stabilized within a few hours to pH 3.4.

β. Sodium carbonate—borax buffer solution was added to the staining solution in the proportion 1 : 1, resulting in an initial pH of 4.5; after 24 hours the pH was 3.4. The proportion 1 : 2 was also used, giving initially pH 2.8 and after stabilization pH 2.5.

The sodium carbonate—borax buffer solution contained:
97.3 ml A (A = 5.30 g Na_2CO_3 calcined at 290°C/l),
2.7 ml B (B = 19.10 g $Na_2B_4O_7 \cdot 10H_2O$/l).
The pH of this buffer solution was 10.7.

The staining time was 24—48 hours. The staining solution was used once only.

b. The nerve fibers were stained by *Bodian's silver staining method* from paraffin sections. Here the method mentioned by Romeis (1943) was employed as such, as well as with the modification that the sections were treated with 99 per cent alcohol for 20 minutes after removal of paraffin (with xylene). From the alcohol they were transferred to Carnoy's solution for 20 minutes and again into 99 per cent alcohol for 20 minutes; thereafter to distilled water and from there into protargol solution, following the method of Romeis. Better success in staining was achieved with this modified version of the staining method.

c. *Hematoxylin-picrofuchsin* (van Gieson, Romeis 1943) *or hematoxylin-eosin staining* (Delafield, Romeis 1943) was used as a general stain.

d. *Feulgen's staining* (Mallory 1944) was employed to establish the presence of desoxyribonucleic acid as a check to the gallocyanin staining.

Fixation was performed with formol and with absolute alcohol-glacial acetic acid solution. The hydrolysis time was 12 minutes after formalin fixation and 6 minutes after alcohol—glacial acetic acid fixation. The staining time in Schiff's reagent was 1 hour.

e. *Methyl green—pyronine staining,* known as the Unna-Pappenheim staining, was employed in order to establish the distribution of ribonucleic and desoxyribonucleic acids in the nerve cells as a check to the gallocyanin staining.

Absolute alcohol-glacial acetic acid solution was used for fixation. The staining solution contained 0.15 g methyl green and 0.25 g pyronine in 2.5 ml 96 per cent alcohol, this solution being diluted to 100 ml with dilute acetate buffer solution. The methyl green was washed with chloroform prior to use. The acetate buffer, of pH 4.4, was diluted with water in the ratio 1 : 3 before use. The sections were left in the staining solution for 20 minutes, whereupon they were quickly rinsed with water. Differentiation was performed in three separate jars of 94 per cent alcohol, 2 minutes in each jar, and the usual dehydration in absolute alcohol.

3. THE RIBONUCLEASE TEST

The ribonuclease method is based on the observation made in 1940 by Brachet, according to which the basophilous granules of the cytoplasm lose their capacity for binding pyronine in Unna-Pappenheim's staining when the tissue sample is treated with an aqueous solution of ribonuclease prior to staining. The nuclear chromatin, on the other hand, retains its stainability with the methyl green component. Brachet inferred from this that the ribonucleic acids are localized in the basophilous granules of the cytoplasm. Similarly it is possible to establish the occurrence of ribonucleic acids in the nucleolus.

In the present work the ribonuclease method of Brachet was employed (Davidson 1953). The tissue sections of 4 μ thickness were kept for 1 hour in distilled water at a temperature of + 37°C, pH 6, to which had been added 0.1 mg of crystalline ribonuclease (salt-free R521, Washington Biochemical Sales Co) per 1 ml. The control sections were kept in the same conditions in distilled water without ribonuclease addition. In both cases the sections were then stained with gallocyanin, according to Feulgen's method, and with methyl green—pyronine.

4. ULTRAVIOLET PHOTOGRAPHY

In the study of the properties of the ganglion cells a comparison was made between ultraviolet photographs of the nerve cells and

the histologic pictures of the same preparation after gallocyanin staining.

The U. V. photographs were taken, using as light source a high pressure mercury arc. Monochromatic radiation was obtained with the aid of a Bausch & Lomb 500 mm grating monochromator with 600 grooves. The quartz condenser of the microscope, manufactured by Cooke, Troughton & Simms, had a numerical aperture of 1.25 (glycerin immersion, n = 1.452). The objective was a 2 mm quartz monochromatic with a numerical aperture of 1.25 (glycerin immersion, n = 1.452), corrected for $\lambda = 2536$ Å. The eyepiece, 10 ×, was of quartz. A fluorescent screen was employed in focussing the image.

C. THE HISTOLOGIC PICTURE PRESENTED BY GALLOCYANIN STAINING

1. THE OBJECT OF THE STAINING

Perusal of the literature relating to the cytochemistry of the nerve cell reveals an obvious connection within the cell between the cytoplasmic nucleic acids and protein synthesis. It is thus possible to draw certain conclusions with regard to the state of the nerve cell from the localization of the nucleic acids and from the content of such acids.

Staining of the tissue with basic gallocyanin—chrome alum lake gives a picture of the basophily of the cell, i.e., primarily of its nucleic acid content. However, the staining fluid may also react with substances other than nucleic acids, depending on the staining conditions. For a correct interpretation of the histologic picture it is necessary to understand the reaction mechanism of gallocyanin-chrome alum staining. This question has been studied in detail by Einarson (1951).

Which of the cellular substances are capable of binding a basic dye?

If the main products of disintegration of *Nissl substance* are considered, it is possible to present its composition roughly as follows, in accordance with Einarson (1951):

- Nissl substance
  - nucleo-protein
    - nuclein
      - nucleic acid
      - protein
    - protein
  - protein (acid)
  - protein (basic)

The cytoplasmic nucleic acid is Feulgen-negative and thus consists of ribonucleic acid.

In the *nucleus* there have been observed (Davidson 1953):
— basic proteins, either protamines or histones,
— proteins with large molecules, such as enzymes, etc.,
— lipins,
— desoxyribonucleic acids,
— ribonucleic acids,
— other phosphorus compounds,
— inorganic substances.

The nucleic acids of the nucleus are mainly desoxyribonucleic acids.

In the nucleus at rest, the bulk of its ribonucleic acid is concentrated in the *nucleolus*. The Feulgen reaction of the nucleolus is negative, except perhaps in its peripheral region, owing to the chromatin associated with the nucleolus. The neutral part of the nucleolus thus consists of ribonucleoproteins, and this centre is possibly surrounded by a desoxyribonucleic acid shell.

The main constituents of the nerve cell are proteins and nucleic acid. In estimating their average amounts, we may take the values which Nurnberger et al. (1952) have calculated from the cell of the anterior horn of the cat, employing the ultraviolet absorption and tissue x-ray technics. The errors inherent in the two methods have been eliminated as far as possible. The figures for the axis-cylinder are supplemented with the result obtained by Engström and Lüthy 1950. Quite naturally there are errors in these figures, owing to the treatment of the tissues; corrections have been made, however, whenever possible by using frozen and desiccated material in the investigations. The average composition of the nerve cell found in this way is illustrated in fig. 5.

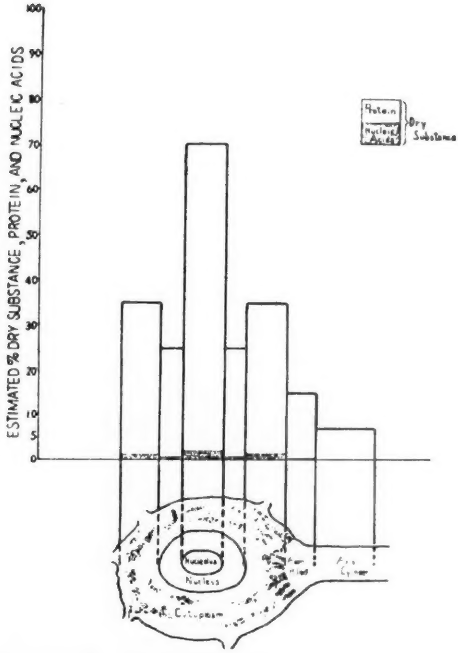


Fig. 5. — The distribution of total dry substance, estimated protein and nucleic acids, in the ventral horn cell of the adult cat. The value for the axis-cylinder is derived from x-ray data in a fresh amphibian axon (Nurnberger et. al. 1952).

These substances, nucleic acids and proteins, maintain basophily in the nerve cell in certain conditions and produce the histologic picture which is obtained with the aid of a basic dye. The basophily of the nucleic acids is due to phosphoric acid groups, but the proteins require certain conditions in order to appear basophilous.

The protein molecule is approximately neutral in isoelectric conditions. The solution of the ampholyte then contains an equal number of cations and anions. Great changes in the dissociation of proteins are brought about by the influence of acids or bases. When acid is added, the increased hydrogen ion concentration diminishes the dissociation of the carboxyl group and the positive charge of the ampholyte gains predominance. Similarly the addition of alkali

reduces the dissociation of the amino group, giving prevalence to the negative charge. The behaviour of the proteins in relation to acids and bases can thus be epitomized in the following statement: *In a solution of pH below the I.P. the protein acts like a base, and in a solution of pH above the I.P. like an acid.* Consequently the protein components of the cell, too, may be the cause of basophily if the pH of the staining solution is higher than the isoelectric point of the protein in question.

A third possibility to account for the binding of the dye is its adsorption by the cell proteins.

2. THE PROPERTIES OF THE GALLOCYANIN STAINING SOLUTION

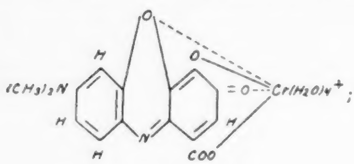
The staining solution used in the gallocyanin staining process is prepared by boiling an aqueous solution of gallocyanin and chrome alum, which results in the formation of a complex compound, the colour lake. According to Einarson (1951), the following components are formed in the solution as products of interionic reactions and, moreover, of hydrolysis:

lake cation,

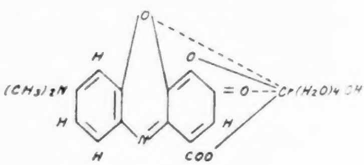
lake sulphate, which is relatively difficultly soluble, and

lake hydroxide, which is practically insoluble in water.

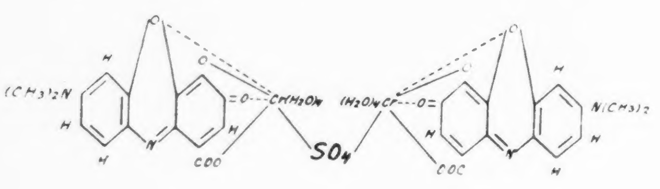
The structural formulae of these components are probably as follows:



Lake-cation



Lake-hydroxide



Lake-sulphate

Lake sulphate is partly dissociated into lake cation and the anion SO_4^{--} and remains partly dissolved in the staining solution as a clear blue component. The rest of the lake sulphate is precipitated, and is separated by filtering together with the lake hydroxide. All OH^- ions produced by hydrolysis are removed from the staining solution, the lake hydroxide being practically insoluble and, furthermore, a very weakly dissociated base. When the lake hydroxide and sulphate have been filtered from the solution prior to its use, no new products of hydrolysis appear for about 4 weeks, after which the staining capacity of the solution is correspondingly reduced. For this reason the staining solution is serviceable for 4 weeks at most.

The actual staining component in this solution is the lake cation, as has been shown by Einarson in his investigations (1951). The lake cation is able to combine by its free + valency with the SO_4^{--} and OH^- ions in the solution or with the basophilous substances in the cell tissue. Lake sulphate is the blue component of the staining solution, the lake cation being reddish, with a resultant violet hue of the staining solution. The tissue is stained an intense blue, the color of the lake sulphate. From this the inference can be drawn that *the lake cations combine selectively with the negative valencies of the nucleoproteins in the cell structure, forming an undissociated, bright blue salt.*

The secondary staining of the tissue is generally quite negligible. The staining appears as a very pale blue, from which the intense blue coloration can be readily distinguished. Since the secondary staining is pale blue, it is evident that it is due to the adsorption of lake sulphate as such, the basic $(CH_3)_2N$ group of the blue lake sulphate being attached to the tissue.

The hydrogen ion concentration of the staining solution can be regulated with the aid of acids or bases. Tests concerning the staining capacity in different pH regions revealed that the pH of the staining solution was only very slowly altered by the addition of a strong acid or base. The staining solution was thus well buffered. Einarson (1951) has conclusively shown, in the form of reaction equations, the reactions produced by the addition of acid or base. On addition of acid, scantily dissociating gallic acid and lake salt may be formed, resulting in a decreased concentration of the lake cation. As a consequence the staining capacity

of the solution diminishes slightly as its acidity increases from its initial value.

On addition of base, very sparingly soluble and weakly dissociating lake hydroxide is formed. This results in a considerable reduction of the specific staining. On the other hand, the intensity of the staining of nerve cells greatly increases with increasing pH, as compared with its initial value. This is exclusively due to the increased adsorption of the lake dye, which is a function of the pH of the solution. The lake hydroxide formed on the addition of base is of significance in that lake hydroxide begins slowly to accumulate with increasing age of the solution, as a blue-violet and sometimes even purplish-red coloration in the tissue.

The lake cation may combine not only with the nucleic acids, but also with the cell proteins. On the basic side of the I.P. of the proteins the negative groups become active, and the dye group with its positive charge can become attached to the protein. It has been found that the proteins combine with the cations of the staining substance on the basic side of their I.P. only. Nissl substance in chromoneutral (maximally staining) nerve cells has an I.P. of about pH 2.7. Thus *binding of the lake cation by protein takes place on the basic side of pH 2.7 only.*

The staining of the cellular substances with gallocyanin-chrome alum solution thus takes the following course:

a. The stain is produced by the selective affinity of the lake cation for the phosphoric acid groups of the polynucleotides in the cell, whereupon a blue nucleic acid—lake salt is formed (Einarson 1947). The binding always involves the positive valency of the lake cation, independent of the type of nucleic acid, i.e., the reaction is the same both with ribonucleic acid and with desoxyribonucleic acid (Einarson 1947, 1949). The staining achieves a maximum in the pH region 1.50—1.75. At this hydrogen ion concentration the staining is *specific for the nucleic acids in the cell,* the adsorption of lake sulphate (non-specific secondary staining) being negligible in this region. When the staining has reached a certain intensity, it does not increase further with continued staining, as the fixation of the color component is a chemical reaction. Thus within a certain pH region the intensity of the staining is dependent on the nucleic acids present at the time of staining only.

The optimum of the specific staining lies at a hydrogen ion

concentration of the solution of pH 1.64. If the pH of the staining solution falls to 0.8 upon the addition of acid, the intensity of the staining also decreases with the decreasing lake cation concentration. With increase of the pH to 4.35 as a result of the addition of base, the intensity of the specific staining decreases as well, since the concentration of the lake cation also diminishes in this case.

b. The binding of the lake cation to the protein components of the cell only takes place within a certain pH region on the alkaline side of the I.P. of the proteins. Since Nissl substance in chromoneutral nerve cells has an I.P. of pH 2.7, gallocyanin—chrome alum staining solution is capable of staining the protein of nerve cells only if the pH of the staining solution exceeds 2.7. This staining is weak in the vicinity of the said I.P. The staining in question is a *secondary staining of the proteins in* the cell. Beside this secondary staining, specific staining also occurs, although to a lesser degree.

c. The adsorption of lake sulphate, which is of an electrostatic character, is caused by the attachment of the $(CH_3)_2N$ group in the dye molecule to the cellular and tissue proteins. The adsorption grows stronger with increasing pH, but an actual increase is observable only from pH 1.84 onwards. This staining is *a non-specific secondary staining of the non-basophilous substances.* Owing to this fact and to the insignificant secondary staining of the proteins the cell itself, and also the background, grow continuously darker, until the maximal total stainability is obtained in the region pH 3.3—3.5. From about pH 3.6 onwards the total staining is abruptly reduced, and at pH 4.3 scarcely any staining of the nerve cells is observable. The background, too, is extremely light in this case.

d. Specific staining occurs in addition to secondary staining in the region pH 1.84—3.8. Below pH 1.84 only specific staining occurs.

e. The gallocyanin—chrome lake cation does not combine with nucleosides or other decomposition products of the mononucleotides. For instance, if the tissue is treated with strong acids, which change the polynucleotides of Nissl substance and split the mononucleotides into sugar phosphate and base, the tissue loses its ability to bind lake cations. If the tissue is treated with alkalis, whereupon the nucleic acids are dissolved or decomposed and the mononucleotides are hydrolyzed to nucleosides as the phosphoric acid is detached, the tissue loses its stainability.

3. INTERPRETATION OF THE STAINING

In order to facilitate the histological interpretation of the slides prepared with the aid of gallocyanin—chrome alum staining, it may be well to summarize the properties of the staining solution, which have been described above in a schematic graphical representation (fig. 6). As datum points, the optimum of the specific staining of the nucleoproteins (pH 1.64) and the isoelectric point (I.P.) of the chromoneutral Nissl substance (pH 2.7) have been chosen. Moreover, the point has been indicated beyond which the influence of electrostatic adsorption is observable (pH 1.84), and the optimum for total staining (pH 3.3—3.5).

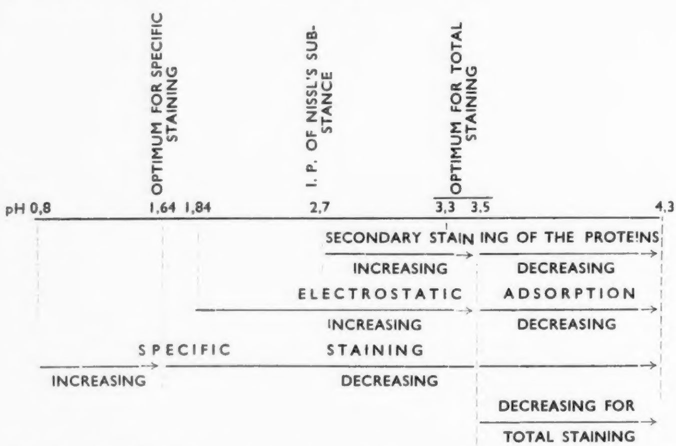


Fig. 6. — The stainability of nerve cells with gallocyanin—chrome alum staining.

It has been mentioned in connection with the description of the staining method that the staining of the sections with gallocyanin—chrome alum was performed at three different hydrogen ion concentrations, namely pH 1.7, pH 2.5, and pH 3.4. Furthermore at pH 3.4, in addition to the standard staining, stainings were also carried out in which the adsorption of dye by the proteins was prevented. In the following we shall discuss the histologic pictures obtained at each of these hydrogen ion concentrations.

pH 1.7. In this staining solution the nucleic acids only are stained and the secondary staining of the proteins is negligible. The histo-

logic pictures are comparatively pale, but clear in their features, and the localization and approximate quantity of the nucleic acids is easily observed under the microscope. No photometric determinations of the intensity of staining were performed.

pH 2.5. Some of the sections were stained in this more alkaline solution in order to extend the observations to include, in part, the proteins of the nerve cell. The original hydrogen ion concentration of the staining solution, pH 1.7, was raised to pH 2.5 with sodium carbonate —borax buffer in the proportion 2 : 1. In this pH region it is mainly specific staining which takes place, but parallel with this also electrostatic adsorption of the dye by the cell proteins. This staining also produced good and useful histologic pictures as a rule, which permitted reliable observation of the changes in the nerve cell.

pH 3.4. The majority of the sections were stained with this even more alkaline staining solution, the pH of which had been raised by means of sodium hydroxide (10 ml 1 N NaOH/100 ml). This solution generally produced very deeply stained preparations, since all three types of staining process take place side by side, namely, specific staining, adsorption, and secondary staining of the proteins. Hence in the nerve cell, part of the proteins are also stained in addition to the nucleic acids. It is difficult to estimate to what extent proteins are present in the picture, the adsorption being incomplete and the chemical binding being dependent upon the I.P. However, this fact is of no great significance from the standpoint of this investigation, as the purpose of this staining, which was carried out at the optimum of total stainability, was to study the state of the entire metabolism of the cell, i.e., the combined occurrence and approximate amount of nucleic acids and proteins. In these pictures the tigrolysis of nerve cells could be observed very well indeed, whereas the sections stained at low hydrogen ion concentration (pH 1.7) only gave positive information on the occurrence of nucleic acids.

If a nerve cell stained in the solution of pH 3.4 is of indefinite shape and dispersed, then it is possible to infer with comparative certainty that protein synthesis in the nerve cell is inadequate to maintain equilibrium with consumption, and the cell is in process of disappearing. It is to be noted, however, that a staining solution of still higher pH may produce an indefinite cell picture of dispersed

appearance in which the stained matter of the nucleus is dispersed and dissolved in its surroundings, even in a normal nerve cell. This observation was made in the course of the experiments relating to the stainability of nerve cells in the different pH regions. It is in accordance with Einarson's (1932) observations. However, no such high pH values of the staining solution (pH 4 or more) were used in this investigation.

In the sections stained in a solution of pH 3.4 other tissues are also stained in addition to nerve cells; in particular, the satellite cells in the ganglia stand out with a very intense stain. This staining is indeed also excellently suited to the study of ganglia in poor condition, in which the neurones are entirely absent and the number of satellite cells is greatly increased.

Some sections were stained in solutions to which a nonionogenic, surface-active substance of polyglycol ether type had been added in order to disperse the lake sulphate and thus to prevent its adsorption. These stainings are the result of a chemical combination of the lake cation with the nucleic acids and part of the proteins. The preparations were as a rule paler than those stained without dispergator, but nevertheless suitable for observations.

4. CHECKING OF THE GALLOCYANIN STAINING WITH RIBONUCLEASE

The reliability of the gallocyanin staining was checked with the aid of the ribonuclease test. The chemical reasoning presented above leads to the conclusion that in the nerve cells gallocyanin at pH 1.7 should stain the nucleotides only. In order to corroborate this, the ribonucleotides were eliminated with the aid of the enzyme ribonuclease, after which staining with gallocyanin was carried out at pH 1.7. The histologic picture obtained in this way was compared with the picture produced by gallocyanin staining under otherwise similar conditions, but without ribonuclease treatment. It was found that in the former case the cytoplasm remained unstained throughout and the nucleolus in part. On the other hand, no noteworthy differences were visible in the staining of the nuclear chromatin. Sections treated with ribonuclease were also stained according to Feulgen's method and with methyl green—pyronine and it was found that the remaining matter in the cell nucleus and in the nucleolus consisted of desoxyribonucleic acid. The cytoplasm

was not stained in the methyl green—pyronine preparation either. Identical pictures of the nerve cell upon ribonuclease treatment were obtained by all three methods: gallocyanin staining, Feulgen's method and methyl green—pyronine, although the picture produced by gallocyanin was the sharpest and the easiest to interpret.

It is evident from the experiment described above that *gallocyanin at pH 1.7 selectively stains the nucleotides only and both the ribonucleotides and desoxyribonucleotides.*

VI. OWN OBSERVATIONS ON THE CHANGES IN THE MYENTERIC (AUERBACH'S) PLEXUS IN INFANTILE HYPERTROPHIC PYLORIC STENOSIS

A. THE ARRANGEMENT OF NERVE CELLS AND GANGLIA

In the series investigated, nerve cells of widely different appearance can be observed in the histologic preparations. These changes occurring in the nerve cells display a certain regularity. According to the changes evident in the nerve cells, they can be classified as belonging to four types, which will be denoted types I, II, III and IV. Similarities are observable on comparison of the phenomena occurring in these cell types with the changes produced experimentally in the nerve cells by Hydén and collaborators, using various stimuli. In the sections stained with gallocyanin at pH 1.7 the presence of nucleic acids is seen. If the staining has been carried out at a higher pH (3.4), the picture will include part of the proteins in addition to the nucleic acids. The histologic preparations of Hydén's school were made at the absorption maximum of the nucleic acids, in which event proteins will also appear in the picture in addition to nucleotides. Thus the two types of picture are comparable with each other. It has been possible, from the histologic pictures, to describe the cytochemical changes revealed by the various cell types.

Since there are different types of nerve cells, it is an obvious inference that there are also ganglia of different grades. The ganglia may be classified into four classes, A, B, C and D. If the nerve cell types and ganglion classes are denoted by their typical properties, it is possible to represent them schematically as follows (fig. 7):

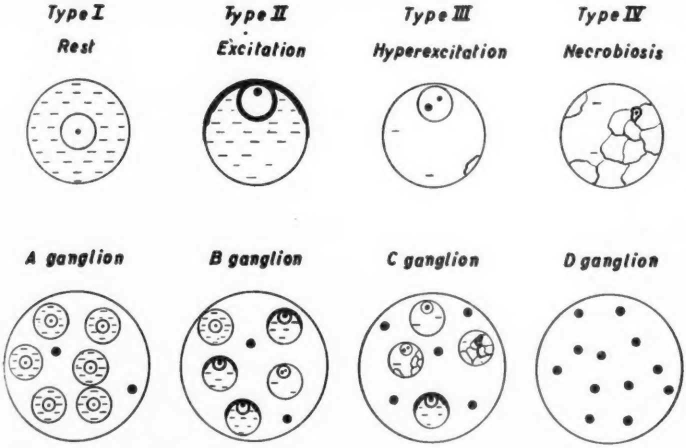


Fig. 7. — Schematic representation of the nerve cell types and ganglion classes.

Type I: The nucleotides are evenly distributed over the cytoplasm. Central nucleus. State of rest of the cell. — Type II: The nucleotides in the cytoplasm are reduced in quantity, the nuclear membrane nucleotides are strongly increased and the nucleolus is increased in size. Eccentric nucleus. State of excitation. — Type III: Tigrolysis in the cytoplasm, nucleotides of the nuclear membrane strongly diminished. State of hyperexcitation. — Type IV: Vacuolization in the cytoplasm, disintegration in the nucleus. Necrobiosis.

A ganglion: Nerve cells of type I. — B ganglion: Nerve cells mainly of types II and III, but also a few nerve cells of type I may occur. — C ganglion: Nerve cells of types III and IV, but also nerve cells of type II may occur. The satellite cells are multiplied. — D ganglion: The nerve cells have vanished, the satellite cells are increased in number.

1. NERVE CELLS

Type I

The shape of the cell and of its nucleus as seen in the section is frequently elliptic, and their longitudinal axes seem to coincide approximately. Circular nuclei also occur, surrounded by a minute cytoplasm. These are obviously circular sections of ellipsoidal nuclei. All kinds of intermediate forms are observable as well. The size of the cells varies on either side of $12 \times 20\ \mu$ and the diameter of the nucleus is of the order of magnitude of $10\ \mu$.

In the nucleus a fairly deeply stained nucleolus is visible, mostly located almost centrally. The basophilous matter occurs dispersed in the nucleus as masses which contrast clearly with their environ-

ment. The remaining part of the nucleus is weakly stained. The cytoplasm is fairly deeply stained and Nissl substance occurs as fine granules evenly distributed throughout the cytoplasm.

Fig. 8 shows a nerve cell of type I from the pure control, case 360/47 P. ventr. The staining was carried out with gallocyanin at pH 1.7; consequently only the substances in the cell which contain nucleic acids have taken up stain. The nuclear chromatin appears as dispersed accumulations in the nucleus. In the vicinity of the nuclear membrane a few very deeply stained masses rich in nucleic acid are seen in the cytoplasm. Nissl bodies are distributed in a finely dispersed state fairly uniformly all over the cytoplasm.

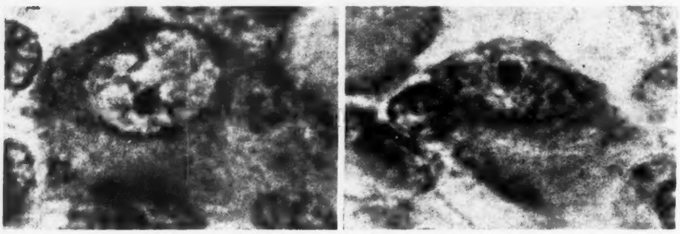


Fig. 8. Fig. 9.

Fig. 8. — Normal nerve cell of type I from pure control case 360/47, P.ventr. Gallocyanin staining, pH 1.7. The nuclear chromatin occurs in scattered masses in the nucleus and Nissl granules are finely divided and distributed all over the cytoplasm. — Magnification × 1500.

Fig. 9. — Nerve cell of incipient type II from the same ganglion as that of fig. 8. The cell type is characterized by hyperchromatism. Slight tigrolysis in the cytoplasm. — Magnification × 1500.

The cell type I has been considered the normal type of nerve cell in its state of rest.

In our series nerve cells resembling type I are also observable, in which the nuclear chromatin is increased and a slight tigrolysis may be noted in the cytoplasm. This process has to be interpreted as a result of increased activity (cf. Hamberger and Hydén 1949a, 1949b). The phenomenon may belong within the range of normal activity of a nerve cell, but it is also possible that it indicates incipient pathologic stimulation. Hydén (1952) has been able, by means of roentgen-microradiographic measurements, to observe even high differences in the nucleoprotein and residual protein content of

normal Purkinje cells in their various stages of activity. This hyperchromatic nerve cell type has been considered a premature cell of type II. Thus probably it constitutes a marginal case between cell types I and II.

In fig. 9 such a hyperchromatic nerve cell is seen, in which the activity is on the increase. The cell has been taken from the same ganglion as the preceding nerve cell of type I. The nucleolus is relatively strong and round. The nuclear chromatin is increased in amount and it is distributed all over the nucleus in small, but distinctly discernible, aggregates. The finely dispersed granules in the cytoplasm are similarly rather deeply stained. Moreover, slight tigrolysis is observable in the central part of the cytoplasm.

Type II

In a nerve cell of type II the nucleus is invariably situated, in longitudinal sections of the cell, at its periphery, and frequently it is rounder in shape. The nucleolus is enlarged and intensely stained. In addition the nuclear membrane is clearly seen, and the chromatin frequently extends from the peripheral part of the nucleus as a deeply stained band to the periphery of the cytoplasm. A highly active nucleolar apparatus is thus observable in the cell. The cytoplasm displays tigrolysis in a greater or lesser degree.

Figs. 10 and 11 represent cell type II. Fig. 10 relates to a case of hypertrophic pyloric stenosis + diarrhea, L 3/46 P.c.mi., and fig. 11 is from the biopsy of case 1317. The cells have been stained with

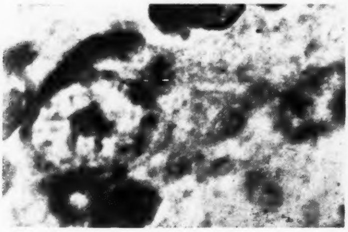


Fig. 10.

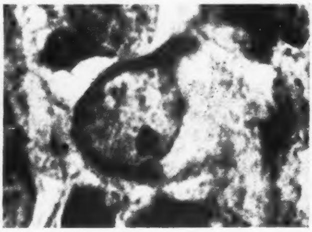


Fig. 11.

Figs. 10 and 11. — Nerve cell of type II. Fig. 10 from hypertrophic pyloric stenosis + diarrhea case L 3/46, P.c.mi; fig. 11 from biopsy 1317. The cells are stained with gallocyanin at pH 1.7 and pH 3.4, respectively. Both cells have a highly active nucleolar apparatus. — Magnification × 1500.

gallocyanin, the first at pH 1.7 and the second at pH 3.4. In both nerve cells a large deeply stained nucleolus is visible, and in the vicinity of the nuclear membrane there is a deeply stained region in the cytoplasm which extends to the periphery of the cytoplasm. The remainder of the cytoplasm is only slightly tigrolytic in fig. 10 and more strongly tigrolytic in fig. 11. In fig. 10 the staining of the chromatin and of Nissl substance is caused by the nucleic acids only, whereas in fig. 11, which is the result of staining at a higher pH, part of the proteins have also been stained in addition to the nucleic acids.

A characteristic feature of the nerve cells of type II is the high production of nucleotides, which is typical, according to Hydén, of nerve cells which have a stimulated protein-producing system (cf. fig. 3b). For this reason the nerve cells of type II have been considered nerve cells in a state of intense activity.

Type III

The nerve cells of type III are characterized by a sparsity of basophilous matter both in the nucleus and particularly in the cytoplasm. Marking the intense activity of the cell there is seen the large nucleolus deeply stained throughout, and very often even two nucleoli and a more or less clearly visible nuclear membrane. As in the preceding type, the nucleus has an eccentric location.

This cell type has been interpreted as a cell in which the activity producing nucleotides and proteins has developed to an extreme, in spite of which the production of these substances is incapable of compensating the immensely increased consumption, the cell being indeed emptied. This consequently represents the state of exhaustion of the cell. In the preparations it is manifested as tigrolysis and chromatolysis (cf. fig. 3c). The tigrolysis and chromatolysis may be of varying degree, depending on how far the evacuation of the cell has advanced (Hydén 1943a).

Nerve cells of this type are frequently somewhat larger in size than those of the preceding types. The largest observed nerve cells of type III were 15 by 27 μ. The nucleus of these cells measured 9 by 13 μ.

Fig. 12 shows a nerve cell of type III from biopsy 1317. The section has been stained with gallocyanin at pH 3.4. As indications of intense activity there are still visible in the cell two very large

and deeply stained nucleoli, and a fairly strong nuclear membrane. Tigrolysis, however, is comparatively far advanced in the cytoplasm and chromatolysis in the nucleus is also clearly distinguishable. The intensity of the staining in the nucleus is not remarkably different from that of the surrounding tissue, but the cytoplasm is lighter in color than its surroundings. It should be noted, in scrutinizing this picture, that in addition to the nucleotides part of the proteins have also been stained (pH 3.4).

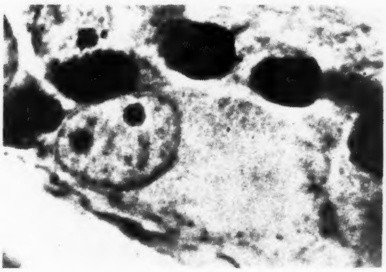


Fig. 12.

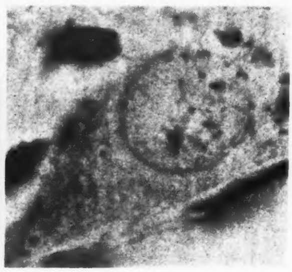


Fig. 13.

Fig. 12. — Nerve cell of type III from biopsy 1317. Gallocyanin staining at pH 3.4. As an indication of intense activity two deeply stained nucleoli and a fairly distinct nuclear membrane are still visible in the cell, but chromatolysis and tigrolysis are also clearly observable. — Magnification × 1500.

Fig. 13. — Nerve cell of type III from autopsy; pyloric stenosis case L 132/47, P.c.ma. Gallocyanin staining, pH 1.7. Far advanced tigrolysis of the cell. Nuclear membrane and nucleoli only faintly visible. — Magnification × 1500.

Fig. 13 is a nerve cell of type III from the autopsy of pyloric stenosis case L 132/47, P.c.ma. It has been stained with gallocyanin at pH 1.7, for which reason the basophilous matter consists almost exclusively of nucleotides. Chromatolysis is fairly far advanced and the nuclear membrane and the two nucleoli are only faintly visible.

Type IV

In the nerve cells belonging to this type signs of necrobiosis are observable: vacuoles in the cytoplasm, shrivelling and disappearance of the nuclear membrane, and disintegration of the nucleus. Frequently the change in the cell seems to commence in such a way that vacuoles are formed in the cytoplasm and with progressive vacuolization the nucleus shrinks and assumes an indefinite,

pycnotic shape. The nucleus is corroded more and more at its periphery and finally the cell disappears altogether. Sometimes an indefinite network of vacuoles is still visible on the site of cytolysed nerve cells (fig. 26, p. 73). The nerve cell type IV is thus characterized by a deformation of the cell. It has been considered a necrobiotic nerve cell.

In fig. 14, from the biopsy series (case 1317) and stained with gallocyanin at pH 3.4, incipient vacuolization is already visible in the cytoplasm of the tigrolytic nerve cell. This picture relates to the same ganglion as figs. 11 and 12.

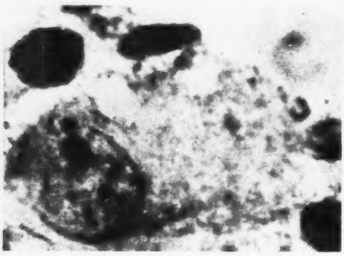


Fig. 14.

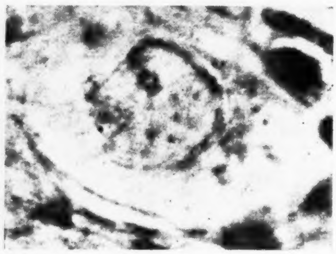


Fig. 15.

Fig. 14. — Nerve cell of type IV from the biopsy series, case 1317. Gallocyanin staining at pH 3.4. Incipient vacuolization visible in the tigrolytic cytoplasm of the cell. — Magnification × 1500.

Fig. 15. — Nerve cell of type IV from biopsy case 1317. Gallocyanin staining at pH 3.4 with dispergator in the staining solution. The vacuolization in the cytoplasm is far advanced with a resultant partial corrosion of the nucleus at its perifery. — Magnification × 1500.

Pertaining to the same case as the preceding, but from a separate preparation, is the nerve cell shown in fig. 15. In this cell the vacuolization in the cytoplasm is already further advanced and the nucleus is partly corroded. The section has been stained with gallocyanin at pH 3.4, in the presence of a dispergator in the staining solution, in order to prevent the adsorption of the dye by the proteins. The staining of the cell chromatin and of Nissl bodies is thus due to chemical combination of the dye with the nucleic acids, and to some extent with the proteins.

Figs. 16 and 17 relate to biopsy case 1234. The staining was performed with gallocyanin at pH 1.7. In fig. 16 the cytoplasm is tigrolytic and the deeply stained nucleus is pycnotic. In the nerve

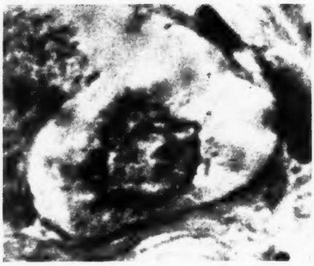
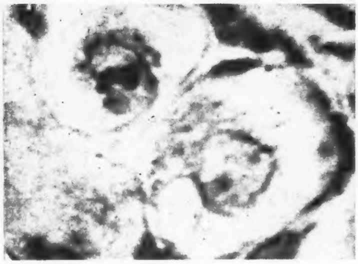


Fig. 16.



Figi 17.

Fig. 16. — Nerve cell of type IV from biopsy case 1234. Gallocyanin staining at pH 1.7. Tigrolysis observable in the cytoplasm; the strongly stained nucleus is pycnotic. — Magnification × 1500.

Fig. 17. — Nerve cells from the same section as that in fig. 16. The cytoplasm is profusely vacuolized, the nucleus shrunken and corroded at its perifery. Of the intense activity of the cells there remain the residues of clear nuclear membranes and in one of the two cells a very deeply stained nucleolus, and two nucleoli in the other cell. — Magnification × 1500.

cells of fig. 17 the cytoplasm is profusely vacuolized and the nucleus is shrivelled and corroded at its edges. The large deeply stained nucleolus in one of the cells and the occurrence of two nucleoli in the other cell, as well as the residues of a strong nuclear membrane, are indications of a previous intense activity of the cell.

2. GANGLIA

The A ganglion

The notation A ganglion has been assigned to normal ganglia. These are frequently narrow, elongated, regular in shape and small in size, although large ganglia of indefinite shape do exist, particularly in cases of pyloric stenosis. The nerve cells are as a rule uniformly distributed throughout the ganglion. The cell types I and incipient II are prevalent. Fig. 18 (p. 66) shows an A ganglion of typical shape from the pure control 360/47, P. ventr., with an abundance of type I cells. Fig. 19 (p. 66) also pertains to an A ganglion of the same pure control case. The nerve cells are of type I, with the exception of the cell in the upper centre, which is a hyperchromatic incipient type II. The nerve cells are fairly evenly distributed as regards their location in the ganglion. In the figure sections of various parts of the cell are also seen. In the upper left

of the figure only the cytoplasm is visible, and the lower cell picture is of a section passing outside the nucleolus. Figs. 38 and 39 (p. 98) are from an A ganglion of the pyloric stenosis + diarrhea case L 3/46, P.c.mi. The nerve cells are of type I. A ganglia occur in every group of the series.

The B ganglion

The nerve cells of B ganglia are mainly of types II and III, but sometimes even cells of type IV may be present to a small extent. Nerve cells of type I are also frequently observable in B ganglia. Such ganglia have also been included in the class of B ganglia, in which the nerve cells are mainly of incipient type II or of type II and the number of normal cells is low. The presence of these nerve cells has to be considered an indication of increased activity of the nerve cells. A B ganglion of this kind is seen in fig. 41 (p. 100), from the pyloric stenosis + diarrhea case L 3/46, P.c.mi. In the figure the ganglion has been divided into two parts. The upper half is characterized by an abundance of nerve cells of incipient type II. At the other end of the ganglion a nerve cell of type II is seen above, and below a nerve cell of type I with a large nucleolus. The B ganglion in fig. 40 (p. 98) is from the same section. All the nerve cells visible in this picture are cells of type II. This ganglion is characterized by a more vigorous activity than that of fig. 41.

Fig. 23 (p. 71) shows a B ganglion from the biopsy series (case 1317), in which the changes are further advanced than in the previously described B ganglia. On the right is a cell of type II. Its nucleolar apparatus is highly active, producing an abundant amount of nucleotides, which are seen as a deeply staining band in the periphery of the cytoplasm. A tigrolytic nerve cell of type III is seen in the upper left part of the figure. In this cell the changes are already further advanced, for scarcely any nucleotides are seen in the cytoplasm. In the lower right-hand corner of this ganglion there is a nerve cell of type IV with incipient vacuolization observable in its tigrolytic cytoplasm. This nerve cell is visible in greater detail in fig. 14 (p. 56).

The B ganglion is thus characterized by an immensely increased production of nucleotides and proteins in the nerve cells. Parallel with production the consumption of these substances is also increased. The changes which can be observed in the nerve cells

vary according to the relative rates of production and consumption; consequently the B ganglia are also of different degrees. The category B ganglia thus covers a range from strongly increased activity in the nerve cells of the ganglion up to the state of exhaustion.

The C ganglion

The structure of the C ganglia is indefinite. This is particularly evident in the biopsy material. The regular structure of the ganglion has disappeared, and nerve cells are situated at random. The ganglia are often of large size and the nerve cells are mainly of types III and IV. Types I and II are seen less frequently. Signs of degeneration and necrobiosis are thus generally observed in the cells of the ganglion. Probably the nerve cells in the C ganglia are hyperstimulated. Furthermore there is an abundance of satellite cells in the C ganglion.

Great difficulty is usually encountered in drawing the line between a B ganglion on the point of transition into the state of exhaustion and a C ganglion. The necrobiotic nerve cell characteristic of a C ganglion is by no means always observable. Therefore it has been necessary to resort to other observations as well in order to reach a conclusion on this point. For instance, where the high content of satellite cells of the ganglion indicates a previous nervous activity of such vigour that nerve cells have obviously disappeared and satelite cells appeared in their place, the ganglia have been classified as C ganglia even when necrobiotic nerve cells are absent or few, or sometimes even when only a single nerve cell in better condition is observable. An abundance of satellite cells has thus to be considered a sign of the degeneration of the ganglion. The C ganglion has probably to be interpreted as a ganglion in a state of exhaustion. Such a ganglion thus differs fundamentally from the B ganglion, which as a rule is characterized by vigorous activity. Quite naturally a great number of marginal cases also occur.

Fig. 21 (p. 70) shows on the right a C ganglion from the biopsy series (case 1317) with an abundance of satellite cells and only a few nerve cells. Near the upper end the ganglion contains a large, pale nerve cell of type III, and on the left there are three pycnotic nuclei, their associated cytoplasm being partially vacuolized. Fig. 24 (p. 72) similarly shows part of a C ganglion from the same case.

In the nerve cells of type IV the nuclei are indefinite and corroded at their periphery, and the cytoplasms are vacuolized.

Fig. 28 (p. 75) also shows a C ganglion of the biopsy series (case 1234). In the nerve cell in the upper right hand a shrunken nucleus with a deeply stained nuclear membrane is seen, as well as tigrolytic cytoplasm. In the upper centre there is a very large, disintegrated nucleus, similarly in a colorless cytoplasm. Fig. 29 (p. 76) shows two of these nerve cells at higher magnification. In fig. 30 (p. 76) part of the second C ganglion of the same case is seen. The profusely vacuolized cytoplasm and the nucleus corroded at its edges are characteristic of the nerve cells of this ganglion. On the left, moreover, a nerve cell of type II is seen, with a clear nuclear membrane and large nucleolus indicating intense activity. Fig. 27 (p. 75) shows a C ganglion of the same case, where the changes are still farther advanced. In the puckered ganglion there only remain two very deformed nerve cells. The ganglion is already changing into a D ganglion.

Fig. 31. (p. 77) also shows a greatly degenerated C ganglion. It is from the biopsy series, case 1612. There are a great number of satellite cells, and in the centre a nerve cell of type IV is seen, with a deeply stained, shrunken nucleus and tigrolytic cytoplasm.

The D ganglion

The characteristic features of the D ganglia are the complete absence of nerve cells, the abundance of satellite cells, and the frequently irregular structure of the ganglion. With the continued changes in the nerve cells the cells ultimately disappear altogether. There are cases in which the sites of the vanished cells are obviously visible. Fig. 25 (p. 73) shows a D ganglion of beautiful shape from the biopsy case 1317, in which the previous location of the nerve cells can still be inferred. In fig. 26 (p. 73), of another D ganglion of the same case, a network of vacuoles is observable; probably an indication of the location of the vanished nerve cells. Fig. 32 (p. 79) shows a D ganglion containing an abundance of satellite cells (case 702). In other D ganglia the change has been more extreme and the entire tissue has been deformed at the same time as the nerve cells have vanished. A typical feature of these ganglia is their indefinite structure.

It is evident that the appearance of D ganglia may also be simulated when, in a ganglion poor in nerve cells, the section traverses a region without nerve cells. Even sections from the edges of a ganglion may suggest a D ganglion by their histologic picture. The frequently observed D ganglia of small size, for instance, are probably of this kind.

B. OBSERVATIONS ON THE DIFFERENT GROUPS OF THE MATERIAL

In this investigation the observations were mainly concentrated on the ganglion classes occurring in the series, since it was possible on this basis to gain a conception of the damage experienced by the nervous system. The changes apparent in the nerve cells and the classification of the ganglia were studied from gallocyanin preparations, but other stains were also employed as an aid, particularly hematoxylin-eosin. Frequently the observations on each of the regions of the pylorus investigated were made from a single good section, since statistically fairly good conformity was obtained in one test series in which the results for several sections from one and the same point were compared. If the sections studied were small, endeavours were made to obtain observations from several sections at the same point, in order to improve the statistical significance of the results. For instance, this was done in the biopsy series, the specimens frequently being of necessity quite small in this group. In such a case the figures in the tables relating to the number of ganglia in the different classes are totals of the figures obtained from all the sections investigated.

1. PURE CONTROL

The control material is divided into two groups, one consisting of cases without any disease of the digestive system, the other of diarrhea cases: The latter group has been included because the autopsy material of the pyloric stenosis cases contained several cases with diarrhea in addition to hypertrophic pyloric stenosis.

The first-mentioned group, or the pure control (table 1), consists of 10 cases, 6 female and 4 male infants. Their age varied

TABLE

PURE CONTROL

Case No.	Sex	Age, days	Duration of illness, days	Clinical diagnosis
310/46 P.ventr.	♂	16	16	Septicemia originating from the navel
310/46 P. dors.	♂	16	16	»
310/46 P.c.mi.	♂	16	16	»
310/46 P.c.ma.	♂	16	16	»
307/47 P.dors.	♀	21	21	Lumbar meningomyelocele Paresis of both lower limbs
307/47 P.c.ma.	♀	21	21	»
152/47	♂	24	24	Mongolian idiocy Subdural hemorrhage
159/45 P.c.mi.	♂	27	27	Premature Icterus neonatorum gravis
159/45 P.c.ma.	♂	27	27	»
112/46 P.ventr.	♀	32	24	Furunculosis Atrophy Bronchopneumonia
112/46 P.dors.	♀	32	24	»
112/46 P.c.mi.	♀	32	24	»
112/46 P.c.ma.	♀	32	24	»
361/17 P.ventr.	♀	58	4	[1]
361/47 P.c.mi.	♀	58	4	[1]
361/47 P.c.ma.	♀	58		[1]

[1] Dead on arrival at the polyclinic

1.

SERIES

Autopsy findings	Ganglion classes								Leuko-cytes, perivasc.	Inflamma-tion of mucoous membrane
	Number				Percentage					
	A	B	C	D	A	B	C	D		
Septicemia originating from the navel	10				100				+	+
»	22				100				+	+
»	7				100				+	+
»	16				100				+	+
Spina bifida Thrombosis of r. renal vein Renal hemorrhages on the r. side Intestinal hemorrhages	5			2	71			29	+	+
»	6				100					
Subdural cerebral hemorrhage Syndactylia	5			1	83			17		
Premature Icterus gravis Anemia of all organs	8				100					
»	8				100					
Necrosis of the skin of the head and of the r. upper arm Bronchopneumonia foci in upper r. and lower l. pulmonary lobes Atrophy of the subcutaneous tissue	10				100				+	
»	10				100				+	
»	7			2	78			22		+
»	10			2	83			17		+
Bilateral pleural empyema Atelectasis of the l. lung Parenchymatous degeneration of the liver Stasis of liver and spleen Lymphadenitis of the neck and mediastinum	11			3	78			22		
»	12			2	86			14		
»	16			9	64			36		

(Continued)

Case No.	Sex	Age, days	Duration of illness, days	Clinical diagnosis
172/47 P.ventr.	♂	88	about 2	Bronchopneumonia
172/47 P.c.mi.	♂	88	about 2	»
360/47 P.ventr.	♀	124	124	Congenital heart disease
360/47 P.c.mi.	♀	124	124	»
360/47 P.c.ma.	♀	124	124	»
157/45 P.c.mi.	♀	151	2	Serous meningitis Rachitis
157/45 P.c.ma.	♀	151	2	»
147/45	♀	196	6	Meningitis

+ very few

between 16 days and 6½ months. The duration of illness had been 2—27 days, except in one case, where it was 4 months. Two patients in the series had pneumonia, two meningitis, one pleural empyema and one septicemia, acute infection having thus been present in six of these ten cases. The other diagnoses were subdural cerebral hemorrhage, icterus neonatorum gravis, vitium cordis congenitum, and spina bifida.

Of the sections used in the study of the pure controls, five were from the ventral wall of the pyloric canal, three from its dorsal wall, seven from the wall of the lesser curvature, and similarly seven from that of the larger curvature, and two sections are from a point which has not been defined in detail. The investigation thus relates to a total of 24 sections. The results are presented in the table on the pure control series, table 1.

At the four points of the pyloric canal investigated the number of ganglia shows the following distribution among the different classes of ganglia:

Autopsy findings	Ganglion classes								Leuko-cytes, perivasc.	Inflamma-tion of mucous membrane
	Number				Percentage					
	A	B	C	D	A	B	C	D		
Atrophy of the subcutaneous tissue Bronchopneumonia of the lower pulmonary lobes Two concrements in the r. renal pelvis	4			1	80			20		
»	3			4	43			57		
Congenital heart disease Interatrial septal defect	20			1	95			5		
»	8			2	80			20		+
»	17			3	65			15		+
Rachitis Serous meningitis	5				100					+
»	13				100					
Purulent leptomeningitis	11			1	92			8	+	+

	A			D		
Ventral wall	92	per	cent	8	per	cent
Dorsal wall	95	»	»	5	»	»
Wall of c. minor	83	»	»	17	»	»
Wall of c. major	86	»	»	14	»	»

The percentage of ganglia of the different classes in the entire region of the pylorus investigated was calculated from the sections taken at the different points of the pyloric canal, the following figures being obtained: A ganglia, 88 per cent and D ganglia, 12 per cent.

The number of D ganglia occurring in the pure control, 12 per cent, has to be taken as the best estimate of the mean value for their simulated occurrence, essentially determined by the location of the section in the ganglion, and this value must be expected to recur in the entire series. The number of D ganglia due to actual changes caused by disease will thus be obtained by deducting 12 per cent from the observed percentage of D ganglia in each case.

A characteristic feature of the pure control are *nerve cells* of type I and incipient type II and the A ganglia composed of such nerve cells, similar in type to those shown in figs. 18 and 19. No B and C ganglia were found in the pure control series.

In the pure control specimens the *nerve fibers* seem to travel in

Fig. 18. — A ganglion from the pure control series, case 360/47, P.ventr. Gallocyanin staining at pH 3.4. The form of the ganglion is typical of an A ganglion. Nerve cells of type I in great numbers. — Magnification × 750.

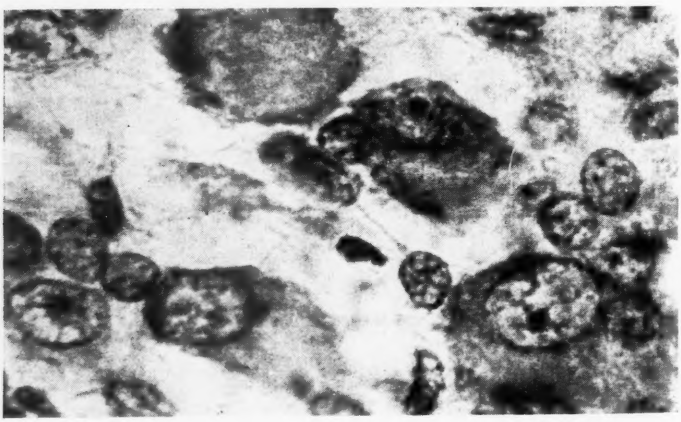


Fig. 19. — Nerve cells of an A ganglion from the pure control series, case 360/47. P. ventr. Gallocyanin staining at pH 1.7. The nerve cells are of type I, except the cell in the upper part of the picture, which is of incipient type II. The picture also shows different nerve cell sections. — Magnification × 1300.

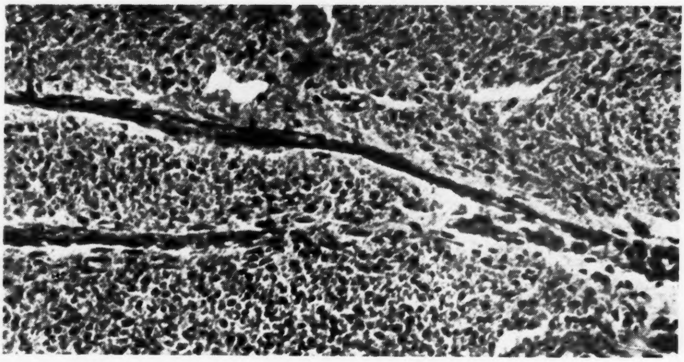


Fig. 20. — Nerve fiber bundle traversing the space between two ganglia from the pure control series, case P 112/46, P.dors. Bodian's stain. — Magnification × 280.

narrow, regular bundles from one ganglion to another. A nerve fiber bundle of this kind is shown in fig. 20, which relates to the case P 112/46.

2. BIOPSIES

The entire series of sections from pyloric stenosis biopsies was fixed in formalin immediately upon operation (Ramstedt's operation). There are 29 cases, including 25 male infants (86.2 per cent) and 4 female infants (13.8 per cent). The age at operation ranges from 3 weeks to 4 months. About 80 per cent of the cases were 4—7 weeks of age. The operation was performed after a period of illness ranging between 9 days and about 3 months. Two infants in this series were prematures with birth weights of 2100 and 2300 g.

The results of this investigation are given in table 2. The distribution of the ganglia among the different classes is as follows: A ganglia 7 per cent, B ganglia 26 per cent, C ganglia 30 per cent, D ganglia 37 per cent.

The changes in the *nerve cells and ganglia* in hypertrophic pyloric stenosis are most distinctly and reliably seen in the biopsy series, the possible influence of other diseases or postmortem changes on the histologic picture being excluded. In the following these changes in the nerve cells and ganglia will be described in the light of a few individual cases.

TABLE

BIOPSY

Case No.	Sex	Age at operation, days	Duration of illness, days	Diagnosis
1693/49	♂	23	9	Hypertrophic pyloric stenosis
702/49	♂	30	10	»
1338/49	♂	33	10	»
3698/48	♂	27	11	»
3321/48	♂	30	11	»
3829/48	♂	40	13	»
1456/49	♂	47	14	»
1342/49	♀	38	17	»
1418/49	♂	57	17	»
777/49	♂	36	18	»
3072/48	♂	29	20	»
173/48	♂	37	21	Hypertrophic pyloric stenosis Harelip
175/48	♂	50	22	Hypertrophic pyloric stenosis
1316/49	♂	44	23	Hypertrophic pyloric stenosis Premature
172/48	♂	43	27	Hypertrophic pyloric stenosis
1317/49	♂	60	27	Hypertrophic pyloric stenosis Premature
1873/49	♂	74	27	Hypertrophic pyloric stenosis
1736/49	♂	42	28	»
3387/48	♂	55	34	»
3031/48	♂	41	41	»
654/49	♂	58	43	»
1022/49	♀	49	49	»
1612/49	♂	53	53	»
626/49	♀	85	53	»
3683/49	♀	64	64	»
763/49	♂	98	68	»
1078/49	♂	94	80	»
1703/49	♂	86	86	»
1234/49	♂	118	97	»

+ + + fairly numerous
+ + some
+ very few

2
SERIES

Ganglion classes								Leukocytes	
Number				Percentage				Perivascul.	Intragangl.
A	B	C	D	A	B	C	D		
		8	16			33	67	++	+++
		7	8			47	53	++	+++
	6	7	6		31	37	32	++	+++
	3	5	3		27	46	27	++	+++
	1	3	3		14	46	43	+	+
		3	2			60	40	+	+
		4	2			67	33	++	++
	2	8	4		14	57	29	++	++
	2	8	4		14	57	29	+	+
	6	16	7		21	55	24	+	+
	2	6	3		18	55	27	+	+++
	1	10	9		5	50	45	+	
	3	4	4		27	37	36	+	
	10	9	8		37	33	30	+	
		5	5			50	50	+	
	9	4	7		45	20	35	+	
	6	9	12		22	33	45		
2	15		5	9	68		23		
	8	2	6		50	12	38	+	
	1	4	3		12	50	38	+	
	3	7	8		17	39	44	+	
3	7	1	8	16	37	5	42	+	
	2	2	6		20	20	60	+	
	3		6		33		67		
6	13	2	7	21	47	7	25		
5	7		4	31	44		25		
10	6	1	10	37	22	4	37	+	
2			2	50			50		
4	6	3	4	24	35	18	23		

A fine example of the changes is afforded by case 1317. The male infant in question was the first child of its parents. The birth weight was 2300 g. Delivery took place 3½ weeks before the calculated time. The mother had hyperemesis throughout the pregnancy. The child began to vomit at slightly over one month of age, and was operated upon after three week's hospitalization, as the vomiting continued in a profuse degree. Distinct peristalsis was noted in the epigastrium. No x-ray investigation was performed in this case, as the diagnosis was considered clear enough without it. The age at operation, 27 days after the onset of symptoms, was 2 months. At pyloromyotomy the wall of the pylorus was found to have a thickness of 3 mm. After operation the vomiting ceased and the weight increased normally.

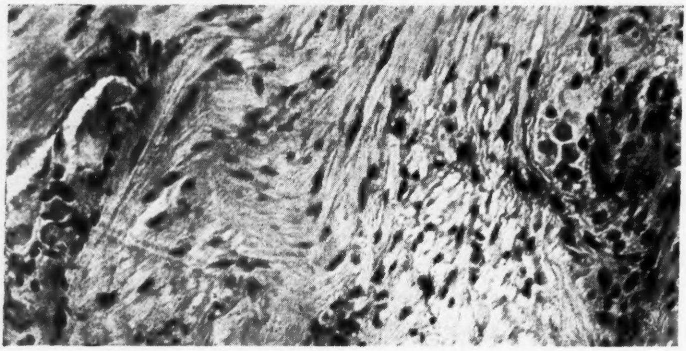


Fig. 21. — Picture from the biopsy series, case 1317. Gallocyanin staining at pH 3.4. On the left, a B ganglion with nerve cells of types II, III and IV. On the right, a C ganglion, in the upper part of which a faintly stained nerve cell of type III is seen, and on the left pycnotic nerve cells of type IV. The satellite cells are increased in number. — Magnification × 300.

In the case in question different types of ganglia from class B to class D can be seen in parallel with each other and the ganglia contain nerve cells of different types side by side. In figs. 21 and 22, A, B, C, and D ganglia are seen in one and the same section. These ganglia will be considered in greater detail at higher magnification.

Fig. 23 shows the B ganglion in this section. It has been stained with gallocyanin at pH 3.4, and consequently part of the proteins have also been stained in addition to the nucleic acids. In this ganglion signs of intense nervous activity are visible. In the nerve cell of type II on the right an intensely active nucleolar apparatus

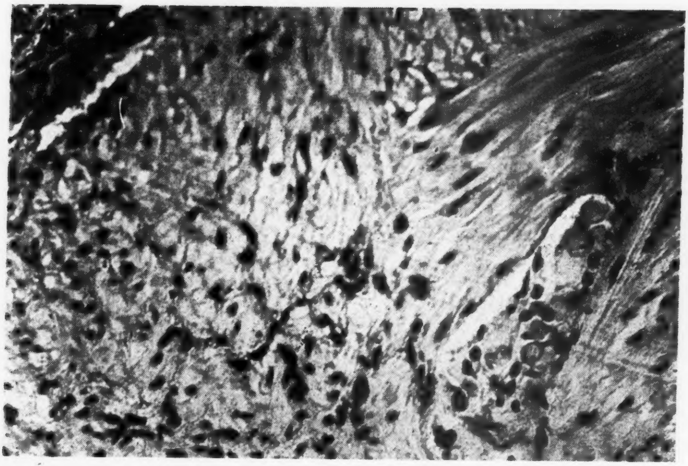


Fig. 22. — From the same preparation as fig. 21. On the right, the same B ganglion as in fig. 21; on the left part of a large D ganglion is visible. — Magnification × 300.

Fig. 23. — Detail of the B ganglion visible in figs. 21 and 22. Gallocyanin staining at pH 3.4. On the left, a tigrolytic nerve cell of type III with two nucleoli as relics of strong activity. On the right, an intensely active nerve cell of type II and in the lower right corner a nerve cell of type IV with incipient vacuolization of the cytoplasm. — Magnification × 1200.

Fig. 24. — C ganglion from the biopsy series, case 1317. Gallocyanin staining at pH 3.4 + dispergator. In the nerve cells of type IV the nuclei are indefinite and corroded at their perifery; the cytoplasms are vacuolized. — Magnification × 1500.

is seen, but in the other nerve cells of the ganglion only a scanty production of nucleotides and proteins is any longer observable. The nerve cell on the left is distinctly tigrolytic. It is of type III. From the existence of two deeply stained nucleoli and the still distinctly discernible nuclear membrane, the inference can be drawn that the activity of the cell has been accelerated to the extreme. However, the chromatolytic nucleus and the strongly tigrolytic cytoplasm clearly indicate that the cell is in a state of exhaustion. The nerve cells in the vicinity of this cell similarly appear poor in chromatin.

The nerve cell at the lower edge of the ganglion, more clearly seen in fig. 14, has to be considered a nerve cell of incipient type IV, since the tigrolytic cytoplasm already displays vacuolization and the cell is thus becoming necrobiotic.

The changes in the nerve cells are still more advanced in the C ganglion of the same case, as is shown in fig. 24. The section has been stained with gallocyanin at pH 3.4 with a dispergator added to the staining solution in order to avoid adsorption of the dye by the proteins. The picture is thus the result of chemical combination of the dye with the nucleic acids and with part of the proteins. The

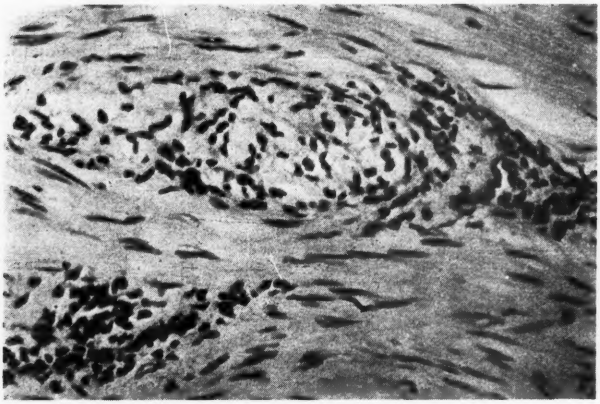


Fig. 25. — D ganglion from the biopsy series, case 1317. Gallocyanin staining at pH 1.7. In the ganglion, with its regular shape and numerous satellite cells. there are sites where nerve cells have obviously disappeared. Magnification × 300.

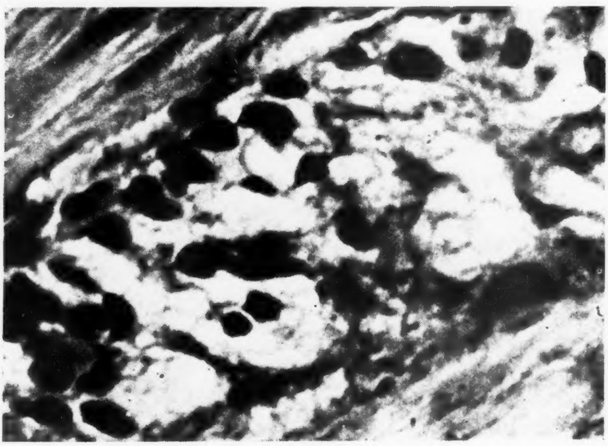


Fig. 26. — Vacuole network in the D ganglion of biopsy case 1317. Numerous satellite cells. Gallocyanin staining at pH 3.4. — Magnification × 1000.

nerve cells are of type IV. The nuclei are of indefinite appearance, corroded at their periphery, and the cytoplasm displays far advanced vacuolization. These nerve cells are necrobiotic.

The case under discussion also contains a great number of D

ganglia, from which the nerve cells have entirely disappeared. A D ganglion of fine shape is seen in fig. 25. In this ganglion with a fairly high number of satellite cells, areas are visible where there may have been nerve cells. Fig. 26, from a different section but from the same case as the D ganglion in fig. 25, shows such areas in greater detail. In this picture a distinct network of vacuoles is visible at the sites of former nerve cells.

The muscle layer in the biopsy specimen is in this case unorganized and strongly hypertrophied.

Case 1234 is that of a male infant, a sixth child. The other children are healthy. The birth weight was 4000 g. The patient had vomited since the age of 3 weeks. On admission to hospital the patient's age was 3½ months and his weight 3500 g. A distinct peristalsis was observable in the epigastrium and palpation indicated a pylorus about the size of a thumb tip. At the x-ray examination a relatively good passage was established, but since the vomiting continued and the weight did not increase, and because the patient could not be admitted to the medical department on account of a shortage of beds, conditions at the patient's home being poor, it was decided to operate on him in order to accelerate his recovery. At the time of operation the illness had persisted for 3 months and 7 days and the age at operation was nearly 4 months. The pyloromyotomy revealed a thickness of the pyloric ring of about 15 mm, and after opening its wall was found to have a thickness of about 6 mm. The muscle appeared to be of slightly softer consistency than is usual in hypertrophic pyloric stenosis. Upon operation the vomiting ceased, and two weeks later the weight was 4230 g.

In this case all classes of ganglia are observable, although A and B ganglia are comparatively abundant. Fig. 27 shows a C ganglion. It has a puckered appearance. Between the folds a couple of deformed residues of nerve cells can be seen with the oil immersion objective.

Fig. 28 shows another C ganglion from the same section. The nuclei of the nerve cells are partly swollen, partly pycnotic. The cytoplasms are invariably tigrolytic. This is most clearly evident in fig. 29, a 1500-fold magnification of two nerve cells of the said ganglion. One of these nerve cells has a swollen nucleus, corroded nuclear membrane and tigrolytic cytoplasm. The other nerve cell has a very deeply stained, shrunken nucleus with intensely stained masses in the cytoplasm in its immediate surroundings. The remainder of the cytoplasm is tigrolytic. The slide was stained with gallocyanin at pH 1.7.

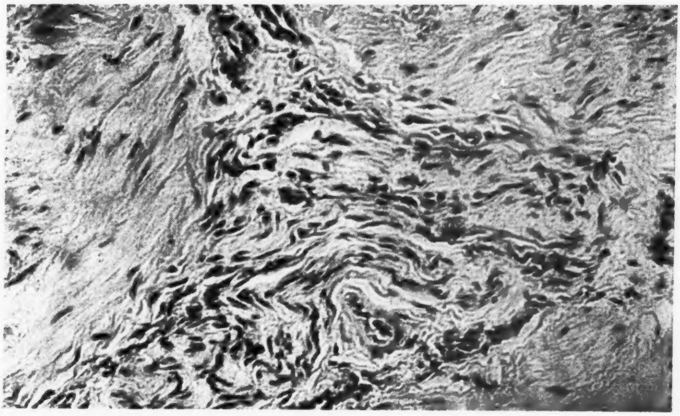


Fig. 27. — C ganglion from biopsy case 1234. Gallocyanin staining at pH 1.7. Puckered structure of the ganglion. Two nerve cells of type IV are visible which have entirely lost their shape. — Magnification × 300.

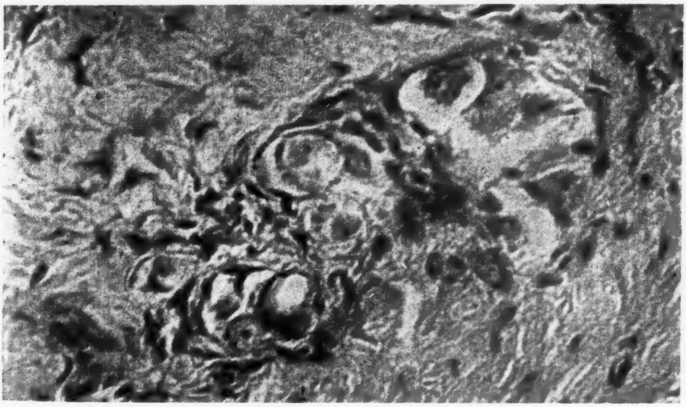


Fig. 28. — C ganglion from biopsy case 1234. Gallocyanin staining at pH 1.7. The progressively changed nerve cells are of type IV. — Magnification × 600.

A further C ganglion of the same biopsy specimen and from the same section as that of fig. 29 is shown in fig. 30. On the right there are two nerve cells of type IV with shrunken nuclei, partly corroded nuclear membranes and largely vacuolized cytoplasm. The very deeply stained nucleolus in one of these cells, and the faint traces

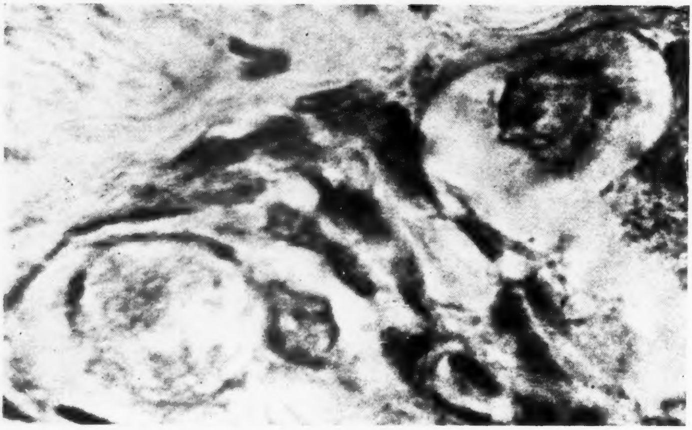


Fig. 29. — Higher magnification of the ganglion in fig. 28. One nerve cell has a deeply stained, shrunken nucleus and a tigrolytic cytoplasm. The nucleus of the other nerve cell is immensely swollen and its nuclear membrane is corroded. Tigrolysis in the cytoplasm. Both nerve cells are of type IV. — Magnification × 1500.

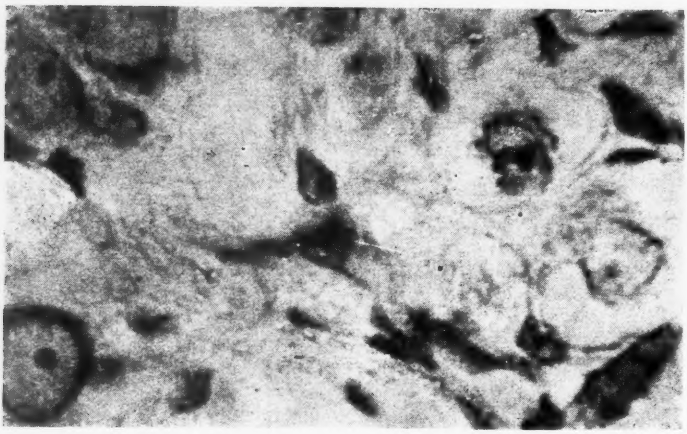


Fig. 30. — C ganglion from biopsy case 1234. Gallocyanin staining at pH 1.7. On the right, two nerve cells of type IV with shrunken nuclei corroded at their perifery and vacuolized cytoplasm. In the lower left part, an intensely active nerve cell of type II. In the upper part obviously young nerve cells in the process of development to active nerve cells. — Magnification × 1500.

of two nucleoli in the other cell are an obvious indication of a previous intense activity of the cells. In the lower left part a nerve cell of type II is visible, with a hyperchromatic nuclear membrane and a deeply stained nucleolus as a sign of its vigorous activity.

This specimen is from the oldest (4 months) of the operated patients in the series in question. The ganglia contain a great number of far advanced necrobiotic nerve cells, but in addition to these also highly active nerve cells of type II and moreover even cells of normal appearance (fig. 30). The latter may be active nerve cells which have developed from the small nerve cells of the ganglion or such cells in process of development (Stöhr 1951). Consequently a ganglion of this kind appears to be undergoing a process of regeneration. In addition to these ganglia, A and B ganglia are also observable in this case in relatively large numbers (together about 60 per cent), so that signs of recovery seem to be noticeable in this case.

In the biopsy series abundant D ganglia are encountered. Likewise there are numerous examples of C ganglia in which the transformation is far advanced. The C ganglion of case 1234 (fig. 27)

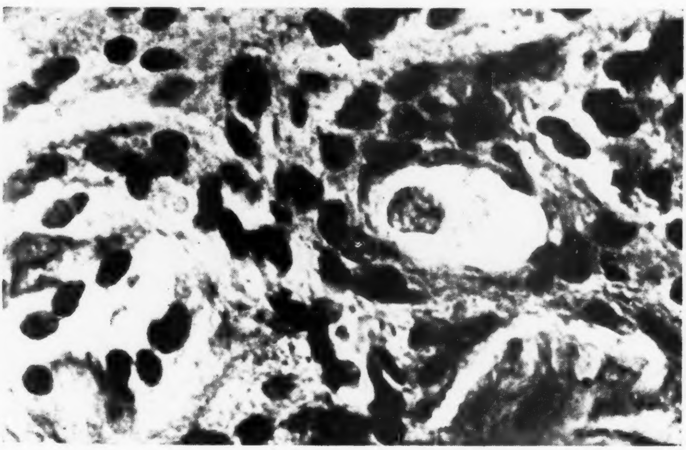


Fig. 31. — C ganglion from biopsy case 1612. Gallocyanin staining at pH 3.4. The satellite cells are very greatly increased in number and the single remaining nerve cell, which is of type IV, has a shrunken nucleus and tigrolytic cytoplasm. On the left a region is visible from which a nerve cell has probably disappeared. — Magnification × 750.

has already been mentioned. It is already changing into a ganglion of class D. In fig. 31, of biopsy 1612, a further example of a far advanced C ganglion is seen. Only a single nerve cell of type IV is left among the greatly multiplied satellite cells. The nucleus of this cell is pycnotic and deeply stained (gallocyanin; pH 3.4). In the cytoplasm far advanced tigrolysis is observed. The left lower edge of the picture shows a point where a nerve cell has probably disappeared by way of vacuolization.

In the case in question the illness had continued for nearly 2 months. The case (1612) is that of a third child, male, with slight vomiting since birth, but frequent profuse vomiting from 3 weeks of age onwards. The birth weight was 3450 g. The weight on admission to hospital, at the age of 33 days, was 3140 g. In the x-ray examination a constriction in the prepyloric part of the stomach and accelerated peristalsis of the stomach was established. The patient was operated on 2 days after admission. The pylorus was the size of man's thumb, a light-colored, glossy lump of 5 mm. wall thickness. Upon operation the vomiting ceased and 9 days later the increase in weight was 260 g. The age at operation was 53 days; the vomiting had continued all the time, having been profuse for about one month.

The C ganglia described above, in which the transformation is far advanced, throw light upon the disappearance of the nerve cells and the formation of D ganglia. In fig. 32 a D ganglion from biopsy specimen 702 is seen. In this the satellite cells have increased considerably in number. The duration of illness has been short, not more than 10 days. In this case only C and D ganglia have been observable, the latter in greater abundance.

Case 702 is a male infant, a third child. The first child had died of pyloric stenosis at the age of 6 weeks (table 3, case L 1150/45). The second child had been normal. Since the age of 3 weeks the patient suffered from projectile vomiting with increasing frequency. The birth weight was 3350 g and weight on admission to hospital, at 3 weeks of age, 3530 g. The age at operation was 1 month; the illness then had a duration of 10 days. At pyloromyotomy the pylorus was found to be of finger-tip thickness. Upon operation the vomiting ceased and the weight increased 670 g in 2 weeks.

On the basis of the foregoing the question presents itself whether the duration of illness has any influence upon the histologic picture. With a view to answering this question, the cases were divided into five groups according to the duration of the illness:

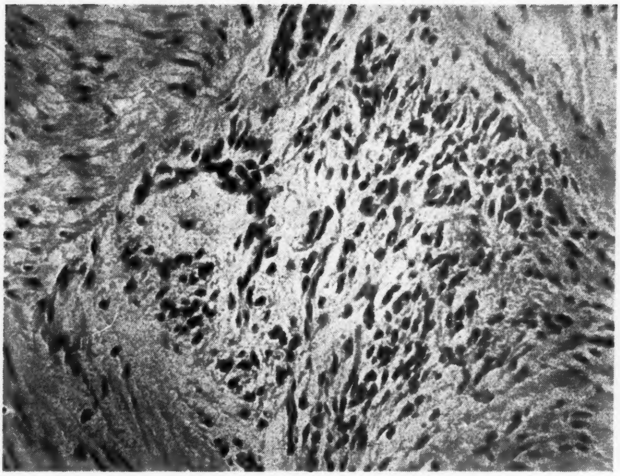


Fig. 32. — D ganglion from biopsy case 702. Gallocyanin staining at pH 3.4. The nerve cells have disappeared and the satellite cells are considerably increased in number. — Magnification × 280.

group I,	duration of illness	less than 10 days	(3 cases,	10 sections)
» II,	» » »	11—20 days	(8 cases,	15 sections)
» III,	» » »	21—30 »	(7 »	, 18 »)
» IV,	» » »	31—50 »	(4 »	, 8 »)
» V,	» » »	51—97 »	(7 »	, 16 »)

The duration of the illness has been reckoned from the appearance of projectile vomiting in those cases where this is stated in the case report. Groups IV and V include five cases in which the time of onset of projectile vomiting has not been accurately stated; the case report only contains the legend: vomiting since birth, which has later developed into projectile vomiting. In these five cases the duration of the illness has been reckoned from birth.

The relative number of ganglia of each class at different stages of the illness is shown graphically in fig. 33. On connecting the points for each class of ganglion, irregular curves are obtained, which give a rough picture of the change in number of the ganglia of each class in the sections with increasing duration of the illness.

It is seen from this graphical presentation that the changes observable in the nerve cells and ganglia have already attained

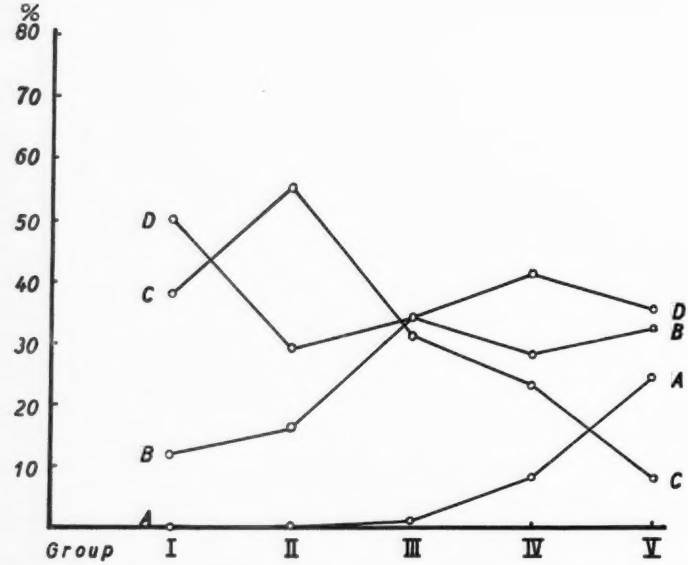


Fig. 33. — The relation between the abundance of the ganglia of different classes and the duration of the illness in hypertrophic pyloric stenosis, in the biopsy series. The illness has been reckoned from the first manifestation of the symptoms of pyloric stenosis.

their maximum when the clinical symptoms of pyloric stenosis have prevailed for 10 days or even for a shorter time (9 days). The changes are clearly greater in groups I and II than in the others. Normal A ganglia are not encountered at all, and even the number of B ganglia is comparatively small, 12—16 per cent. On an average about one half of all ganglia are progressively transformed C ganglia in these groups. Admittedly the D ganglia are more abundant in group I than the C ganglia, but this is possibly accounted for by the fact that only three cases were available and that it is frequently difficult to distinguish sections resembling D ganglia from the actual histologic picture of a D ganglion. In group II the number of D ganglia drops to about 30 per cent and remains between 30 and 40 per cent throughout the rest of the series. If the D ganglia attributable to the location of the section in the ganglion, etc., are deducted, their number being about 12 per cent according to the control series, we obtain for the number of D ganglia produced by

the disease itself about 20—30 per cent. This number of D ganglia thus seems to retain a nearly constant level in the entire series; it indicates that the most extensively transformed D ganglia are no longer capable of regeneration.

Since the maximum changes in the nerve cells as well as the ganglia are already apparent in group I, it seems evident that the clinical symptoms of the disease do not become manifest before the changes in the nerve cells and in the ganglia have attained a certain degree. There is no change in the extent of the lesion during the next few weeks with continuing disease. Only after the condition has lasted more than three weeks do signs of recovery begin to appear: A ganglia begin to occur in the series and their number increases fairly steadily, so that it already exceeds 20 per cent in cases where the duration of the illness has been 2—3 months. The number of B ganglia similarly shows a steady increase, ultimately attaining a value of about 30 per cent. The C ganglia diminish abruptly; after an illness of 2—3 months' duration their number is less than 10 per cent.

In this way the condition of the nerve cells and ganglia experiences a continuous improvement during the course of the disease, but even after 3 months of illness distinct signs of damage are still observable in the nervous system. The recovery is not yet complete this time.

In the cases belonging to groups I and II the boundary between the muscle layers is very indistinct and nervous tissue occurs in disarrangement on the interface of the two muscle layers as well as in the longitudinal muscle layer (fig. 34). Nervous tissue also occurs in the circular muscle layer. These cases are characterized by an abundance of satellite cells in the ganglia, which indicates far advanced changes in the ganglion, and by a scantiness of nerve cells. In group I several ganglia are of a kind in which only a few isolated, small nerve cells can be found among the numerous satellite cells. In groups II and III tigrolytic nerve cells are encountered in great numbers and there are sometimes also necrobiotic cells among them. Even in groups IV and V the nerve cells in the ganglia are rather few, but most of them appear normal.

The *nerve fiber bundles* are hypertrophied and strongly dispersed in the biopsy series. In some single nerve fibers thickened portions can be observed.

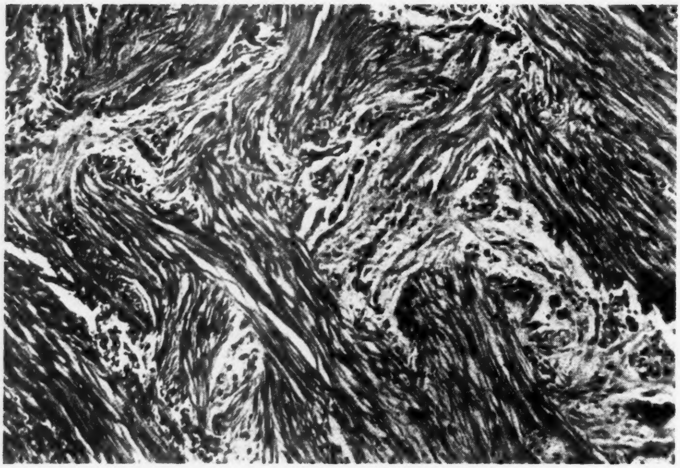


Fig. 34. — Muscle bundles exhibiting the irregular course typical of hypertrophic pyloric stenosis; between them ganglia in disarray. Picture from biopsy case 3072/48. Hematoxylin-eosin staining. — Magnification × 100.

With regard to *leukocytes* the following observations have been made: In group I, where the duration of the disease has been 9—10 days, neutrophil granulocytes are fairly abundant, and als olymphocytes in some amount. They are situated around blood vessels and in quite considerable numbers in the ganglia. In group II the number of leukocytes is less than in the preceding group. Conditions are also similar in group III. In cases of longer duration of the disease, i.e., in groups IV and V, only very few leukocytes and lymphocytes can be observed (cf. table 2).

3. AUTOPSIES—PYLORIC STENOSIS WITHOUT DIARRHEA

The autopsy series relating to pyloric stenosis not complicated by diarrhea consists of 10 cases, seven of them male, and three female infants. The duration of the illness in these patients has been from 9 days to 3 months, the age at death being 1—3 months. The cause of death was in five cases cachexia due to hypertrophic pyloric stenosis, in five cases pneumonia, in one sepsis and in one case congenital heart disease.

The investigation has been made with 28 specimens taken from

the region of the pyloric canal, six of them from the ventral wall, seven from the dorsal wall, eight from the wall of the lesser curvature, and seven from the wall of the greater curvature. The results of these investigations are given in the table on the autopsies of pyloric stenosis cases, table 3.

The distribution of the ganglia according to class, in the different areas of the pyloric canal was as follows:

	A	B	C	D
Ventral wall	4 per cent	31 per cent	50 per cent	15 per cent
Dorsal wall	2 » »	12 » »	60 » »	26 » »
Wall of c. minor	1 » »	16 » »	67 » »	16 » »
Wall of c. major	7 » »	19 » »	50 » »	24 » »

It is seen from the foregoing that no substantial differences exist between the different areas of the pylorus with regard to the number of ganglia of the different classes. In the entire region of the pyloric canal the ganglia investigated show the following distribution:

A ganglia 3 per cent, B ganglia 19 per cent, C ganglia 57 per cent, D ganglia 21 per cent.

The relation between the relative number of ganglia of each class and the duration of the disease is seen from the graph (fig. 35), with the percentage occurrence of each ganglion class as ordinates and the groups of different duration of the disease marked on the abscissa. There are five of these groups:

Group	I,	duration of illness less than	10 days	(1 case,	1 section)	
»	II,	» » »	11—20 days	(2 cases,	7 sections)	
»	III,	» » »	21—30 »	(3 » ,	5 »	)
»	IV,	» » »	31—50 »	(1 case,	4 »	)
»	V,	» » »	51—100 »	(3 cases,	11 »	)

It is seen from this graph that even in this series the changes in the nerve cells and in the ganglia have already attained their maximum when the disease has continued for about 10 days. In the initial stage of the disease the cases present C and D ganglia only, the first-mentioned constituting the major part, about 80 per cent, and the remainder, about 20 per cent, being D ganglia. This condition broadly speaking prevails in all cases with a duration of the disease not exceeding one month. From the time of one month

TABLE

PYLORIC STENOSIS

Case No.	Sex	Age, days	Duration of illness, days	Clinical diagnosis
132/47 P.c.ma.	♂	30	9	Hypertrophic pyloric stenosis Bilateral bronchopneumonia Atrophy
P 13/46 P. ventr.	♀	35	14	Hypertrophic pyloric stenosis
P 13/46 P.dors.	♀	35	14	»
P 13/46 P.c.mi.	♀	35	14	»
838/46 P.ventr.	♂	52	20	Hypertrophic pyloric stenosis Bronchopneumonia foci
838/46 P.dors.	♂	52	20	»
838/16 P.c.mi.	♂	52	20	»
838/46 P.c.ma.	♂	52	20	»
L 881/45 P.c.mi.	♂	30	23	Hypertrophic pyloric stenosis Atrophy
L 881/45 P.c.ma.	♂	30	23	»
P 253/46 P.ventr.	♂	55	24	Hypertrophic pyloric stenosis Atrophy Acute gastroenteritis
P 253/46 P.dors.	♂	55	24	»
P 253/46 P.c.mi.	♂	55	24	»
P 253/46 P.c.ma.	♂	55	24	»
L 128/47 P.c.mi.	♂	41	25	Hypertrophic pyloric stenosis Melena
L 128/47 P.c.ma.	♂	41	25	»
P 337/45 P.ventr.	♂	59	27	Hypertrophic pyloric stenosis Hemorrhagic duodenitis
P 337/45 P.dors.	♂	59	27	»
P 337/45 P.c.mi.	♂	59	27	»

3

AUTOPSY SERIES

Autopsy findings	Ganglion classes								Leuko-cytes, perivasc.	Inflamma-tion of mucous membrane
	Number				Percentage					
	A	B	C	D	A	B	C	D		
Hypertrophic pyloric stenosis Pneumonia foci			4	1			80	20	+	++
Hypertrophic pyloric stenosis Anemia of all organs Atrophy of the subcutaneous tissue			13	1			93	7	+	+
»			7	1			88	12	+	+
»			3				100		+	+
Hypertrophic pyloric stenosis Bronchopneumonia foci			22	2			92	8	+	+
»	1		12	10	5		52	43	+	+
»			14	4			78	22	+	+
»			16	7			70	30	+	+
Hypertrophic pyloric stenosis Anemia of all organs Atrophy of the subcutaneous tissue Pulmonary hypostasis			13	2			87	13	+	+
»			17	1			94	6		+
Hypertrophic pyloric stenosis Catarrhal enteritis Atrophy of the subcutaneous tissue Anemia of all organs		4	3			56	44			+
»			10	2			83	17	+	
»			9	2			82	18	+	+
»			16	2			89	11	+	
Hypertrophic pyloric stenosis Bronchopneumonia of the lower l. and upper r. lobes			4	3			57	43	+	
»			2	4			34	66	+	
Hypertrophic pyloric stenosis Hemorrhagic duodenitis			7	11			61	39	++	++
»			7				100		++	+++
»			4	4			50	50	++	++

+++ fairly numerous ++ some + very few

(Continued)

Case No.	Sex	Age, days	Duration of illness, days	Clinical diagnosis
P 337/45 P.c.ma.	♂	59	27	Hypertrophic pyloric stenosis Hemorrhagic duodenitis
L 66/45	♂	46	28	Hypertrophic pyloric stenosis Acute gastroenteritis Intoxication
296/47 P.ventr.	♂	57	28	Hypertrophic pyloric stenosis Acute gastroenteritis
296/47 P.dors.	♂	57	28	»
296/47 P.c.ma.	♂	57	28	»
394/47 P.c.ma.	♀	51	30	Acute gastroenteritis Intoxication Hypertrophic pyloric stenosis
140/47 P.dors.	♂	66	30	Hypertrophic pyloric stenosis Sepsis
P 228/46 P.ventr.	♂	52	31	Post-operative state after hypertrophic pyloric stenosis Acute gastroenteritis
P 228/46 P.dosr.	♂	52	31	»
P 228/46 P.c.mi.	♂	52	31	»
P 228/46 P.c.ma.	♂	52	31	»
L 750/46 P.ventr.	♂	45	32	Hypertrophic pyloric stenosis
L 750/46 P.dors.	♂	45	32	»
L 750/46 P.c.mi.	♂	45	32	»
L 750/46 P.c.ma.	♂	45	32	»
L 1150/45 P.ventr.	♂	42	33	Hypertrophic pyloric stenosis Acute gastroenteritis Intoxication

Autopsy findings	Ganglion classes Number A	B	C	D	Percentage A	B	C	D	Leuko-cytes, perivasc.	Inflamma-tion of mucous membrane
Hypertrophic pyloric stenosis Hemorrhagic duodenitis	1		15	9	4		60	36	++	+++
Hypertrophic pyloric stenosis Acute gastroenteritis			13	3			81	19	+	+
Hypertrophic pyloric stenosis Catarrhal enteritis Bilateral stasis of lower pulmonary lobes Atrophy of the subcutaneous tissue			8	5			61	39		
»			3	2			60	40		
»	1		4	10	6		27	67	+	+
Hypertrophic pyloric stenosis Catarrhal enterocolitis Parenchymatous degeneration of the liver Renal stasis Atrophy of the subcutaneous tissue			17	14			80	20		++
Hypertrophic pyloric stenosis Meningeal edema Adipose degeneration of the liver			10	2			83	17		
Hypertrophic pyloric stenosis Sutured incision wound in the serous membrane and incision wound in the musculature of the pylorus Interintestinal fibrous adhesion Atrophy of the subcutaneous tissue Hepatic stasis	7			1	80			20	+	++
»		16	1	2		84	5	11	++	++
»		5				100			+	++
»	1	8			11	89			+	+
Hypertrophic pyloric stenosis		12		3		80		20	+	+
»	1	7	3	2	8	54	23	15	+	
»		4	3	1		50	38	12		
»	3	13	2	5	12	57	8	22	+	
Hypertrophic pyloric stenosis			19	7			73	27		

(Continued)

Case No.	Sex	Age, days	Duration of illness, days	Clinical diagnosis
L 1150/45 P.dors.	♂	42	33	Hypertrophic pyloric stenosis Acute gastroenteritis Intoxication
L 1150/45 P.c.ma.	♂	42	33	»
P 334/46 P.ventr.	♂	51	33	Hypertrophic pyloric stenosis Acute gastroenteritis Intoxication
P 334/46 P.dors.	♂	51	33	»
P 334/46 P.c.mi.	♂	51	33	»
P 334/46 P.c.ma.	♂	51	33	»
L 214/46 P.c.mi.	♂	43	34	Hypertrophic pyloric stenosis Gastroenteritis
L 214/46 P.c.ma.	♂	43	34	»
L 3/46 P.ventr.	♂	41	41	Hypertrophic pyloric stenosis Acute hemorrhagic gastritis
L 3/46 P.dors.	♂	41	41	»
L 3/46 P.c.mi.	♂	41	41	»
L 3/46 P.c.ma.	♂	41	41	»
410/45 P.ventr.	♂	66	42	Hypertrophic pyloric stenosis Acute gastroenteritis Intoxication
410/45 P.dors.	♂	66	42	»
410/45 P.c.mi.	♂	66	42	»
410/45 P.c.ma.	♂	66	42	»
P 430/45 P.ventr.	♂	68	54	Hypertrophic pyloric stenosis Acute hemorrhagic gastritis Intoxication

Autopsy findings	Ganglion classes								Leuko-cytes, perivasc.	Inflamma-tion of mucous membrane
	Number				Percentage					
	A	B	C	D	A	B	C	D		
Hypertrophic pyloric stenosis			12	5			67	33		
»		6	9	9		26	37	37		
Hypertrophic pyloric stenosis Hypertrophic colitis Atrophy of the subcutaneous tissue Gastritis			12	4			75	25	+	++
»			11	8			58	42	+	+
»			24	11			68	32	+	++
»			15	14			52	48	+	+
Hypertrophic pyloric stenosis Hemorrhagic enteritis Stasis of liver and kidneys Atrophy of the subcutaneous tissue	5	2	5	6	28	11	28	33		+
»		11	7	4		50	32	18	+	+
Hypertrophic pyloric stenosis Catarrhal gastroenterocolitis Atrophy of the subcutaneous tissue Stasis of liver and kidneys			8	6			57	43	+	+
»	3	3	3	15	12	12	12	64	+	+
»			4	7			36	64		+
»		2	10	4		12	63	25	+	+
Hypertrophic pyloric stenosis Enteritis Stasis of liver and kidneys Atrophy of the subcutaneous tissue		64		5	9			36	+	
»		33		10	5			67	+	
»		35		11	6			65	+	
»		36		9	5			64		
Hypertrophic pyloric stenosis Catarrhal enterocolitis Adipose degeneration of the liver Renal stasis Atrophy of the subcutaneous tissue		14		6	1			86	+	

(Continued)

Case No.	Sex	Age, days	Duration of illness, days	Clinical diagnosis
P 430/45 P.dors.	♂	68	54	Hypertrophic pyloric stenosis Acute hemorrhagic gastritis Intoxication
P 430/45 P.c.ma.	♂	68	54	»
P 30/46 P.ventr.	♂	75	54	Hypertrophic pyloric stenosis Acute enteritis Atrophy
P 30/46 P.dors.	♂	75	54	»
P 30/46 P.c.mi.	♂	75	54	»
P 30/46 P.c.ma.	♂	75	54	»
P 286/46 P.ventr.	♂	76	55	Hypertrophic pyloric stenosis Acute gastroenteritis
P 286/46 P.dors.	♂	76	55	»
P 286/46 P.c.mi.	♂	76	55	»
L 286/46 P.c.ma.	♂	76	55	»
L 503/46 P.ventr.	♂	70	56	Hypertrophic pyloric stenosis
L 503/46 P.dors.	♂	70	56	»
L 503/46 P.c.mi.	♂	70	56	»
L 503/46 P.c.ma.	♂	70	56	»
P 354/45 P.ventr.	♀	83	83	Congenital heart disease Hypertrophic pyloric stenosis
P 354/45 P.dors.	♀	83	83	»
P 354/45 P.c.mi.	♀	83	83	»
P 354/45 P.c.ma.	♀	83	83	»

Autopsy findings	Ganglion classes								Leuko-cytes, persivasc.	Inflamma-tion of mucous membrane
	Number				Percentage					
	A	B	C	D	A	B	C	D		
Hypertrophic pyloric stenosis Catarrhal enterocolitis Adipose degeneration of the liver Renal stasis Atrophy of the subcutaneous tissue			5	3			63	37		+
»			10	1			99	1		+
Hypertrophic pyloric stenosis Catarrhal enteritis Atrophy of the subcutaneous tissue Cerebral edema		1	4	4		12	44	44	+	+
»			5	4			56	44	+	
»	1		17	19	4		63	33	+	
»			12	13			80	20	+	+
Hypertrophic pyloric stenosis Catarrhal gastroenterocolitis Stasis of liver and kidneys Atrophy of the subcutaneous tissue			8	3			73	27		
»			2	5			29	71		+
»			5	3			63	37		++
»			11	5			69	31		+
Hypertrophic pyloric stenosis	3	7			30	70			+	+
»		3	2			60	40		+	+
»	1	10	5		6	62	32		+	+
»	4	7		2	25	58		17	+	+
Congenital heart disease Hypertrophic pyloric stenosis	1	2	10	7	5	10	50	35	+	++
»			23	9			72	28	+	++
»			9	1			90	10	+	+
»			12	6			67	33	+	+

(Continued)

Case No.	Sex	Age, days	Duration of illness, days	Clinical diagnosis
368/47 P.ventr.	♂	98	84	Hypertrophic pyloric stenosis Acute gastroenteritis Intoxication Pneumonia of the r. lung
368/47 P.dors	♂	98	84	»
368/47 P.c.mi.	♂	98	84	»
368/47 P.c.ma.	♂	98	84	»
L 1123/45 P.ventr.	♀	89	89	Hypertrophic pyloric stenosis
L 1123/45 P.dors.	♀	89	89	»
L 1123/45 P.c.mi.	♀	89	89	»
L 824/45 P.dors.	♂	114	100	Hypertrophic pyloric stenosis Pneumonia of the r. lung
L 824/45 P.c.mi.	♂	114	100	»
L 824/45 P.c.ma.	♂	114	100	»
L 164/45 I	♀	151	114	Hypertrophic pyloric stenosis Abscess in the gluteal region on the r. side with osteomyelitis of the ilium Septicemia Acute myocarditis Hemorrhagic gastritis
L 164/45 II	♀	151	114	»
L 164/45 III	♀	151	114	»
L 164/45 IV	♀	151	114	»

Autopsy findings	Ganglion classes								Leuko-cytes, perivasc.	Inflamma-tion of mucous membrane
	Number				Percentage					
	A	B	C	D	A	B	C	D		
Hypertrophic pyloric stenosis Catarrhal enteritis Parenchymatous degeneration of the liver Bronchopneumonia of the upper r. lobe			7	4			64	36	++	++
»			7	3			70	30	+	++
»			2				100			++
»			9	4			69	31	++	++
Hypertrophic pyloric stenosis		10	6	2		55	8	37		
»		3	9	4		19	25	56	+	+
»			7	3			30	70	+	+
Hypertrophic pyloric stenosis Pneumonia of the r. lung Hemorrhagic gastritis			9	5			64	36		+
»			10	3			77	23	+	+
»			10	2			83	17	+	+
Hypertrophic pyloric stenosis Abscess in the gluteal region on the r. side with osteomyelitis of the ilium Acute myocarditis Hemorrhagic gastritis	1	3			25	75			+	+
»	4	2		1	57	29		14	+	
»		4	2	3		45	22	33	+	+
»	1	3	1		20	60	20		+	+

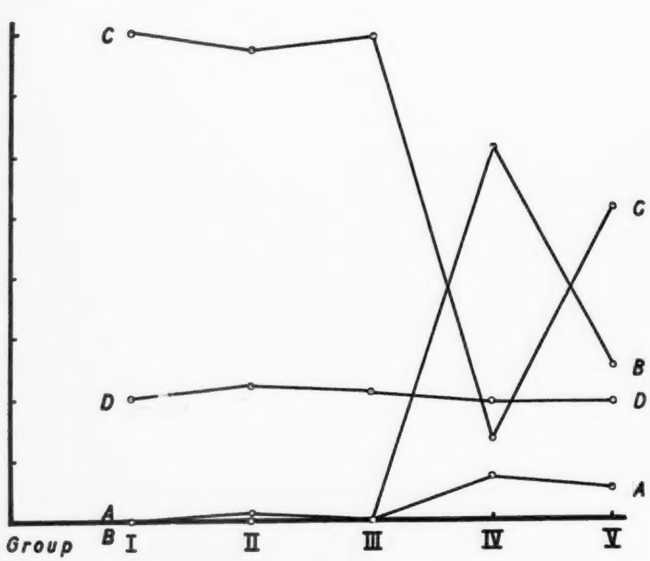


Fig. 35. — The abundance of the ganglia of different classes in relation to the duration of illness in hypertrophic pyloric stenosis, in the autopsy series uncomplicated by diarrhea. The illness has been reckoned from the first manifestation of the symptoms of pyloric stenosis.

onwards signs of recovery are observed. A ganglia begin to appear, which have been almost entirely absent up to then. The B ganglia, which are absent at the beginning of the disease, also begin to increase simultaneously with the A ganglia when the disease has lasted more than one month. The C ganglia then show a corresponding decrease. It is probable that the C ganglia are transformed into the better condition of B ganglia. As late as 3 months after the onset of the disease about 50 per cent of C ganglia can be observed. The number of A and B ganglia has then increased to about 30 per cent. The number of D ganglia remains constant throughout the entire period of illness investigated (more than 3 months). Thus it seems obvious that the D ganglia are not capable of regeneration.

As an illustration of the changes in this series we shall choose the case L 132/47. The patient, a male infant, is a second child. The first child was healthy. Birth weight was 3120 g. From about three weeks of age the patient had had projectile vomiting immediately after every meal. After one week's illness the patient was admitted to the hospital. The weight

Fig. 36. — B ganglion in the process of transformation to a C ganglion; from the pyloric stenosis autopsy series, case L 132/47, P.c.ma. Gallocyanin staining at pH 1.7. The nerve cells are all of type III. The ganglion is in a state of exhaustion. The satellite cells are increased in number. In the lower right part, small nerve cells which would possibly have developed into active cells. — Magnification $\times$ 1000.

was then 2440 g. He was atrophic and pale; the fontanel was hollow. In the hospital the patient grew continuously worse. Vomiting was profuse and exitus ensued on the third day in hospital.

The autopsy findings were hypertrophic pyloric stenosis and pneumonia foci in both lungs. At the time of death the infant was one month old, and the projectile vomiting had lasted 9 days.

Fig. 36 shows a B ganglion in the process of transformation to a C ganglion from this case L 132/47. In this the changes are already comparatively far advanced. The nerve cells are of type III and consequently in a state of exhaustion. Satellite cells are observed in abundance. On the lower right in the figure small nerve cells are also visible, which might have developed into active nerve cells (Stöhr 1951).

The *nerve fibers* occur in large bundles in this series, as in the biopsy series.

Leukocytes are observable perivascularly and in some cases even in the ganglia. Here and there in the mucous membrane slight inflammation is also encountered.

4. AUTOPSIES—PYLORIC STENOSIS WITH DIARRHEA

The series investigated includes 17 autopsies of cases with hypertrophic pyloric stenosis complicated by diarrhea, 15 of which are male and 2 female infants. In these cases the symptoms of pyloric stenosis had persisted between 3 weeks and nearly 4 months. The age at death was between 6 weeks and about 5 months. The cause of death was diarrhea in 16 cases and pneumonia in one case.

The investigations relating to this material have been made with 56 specimens, 12 of them taken from the ventral wall, 13 from the dorsal wall, 11 from the wall of the lesser curvature, and 15 from the wall of the greater curvature. Five specimens of two cases have not been defined as to their location.

The distribution of the ganglia according to class in the different areas of the pyloric canal was as follows:

	A	B	C	D
Ventral wall	5 per cent	3 per cent	56 per cent	36 per cent
Dorsal wall	2 » »	11 » »	51 » »	36 » »
Wall of c. minor	4 » »	4 » »	55 » »	37 » »
Wall of c. major	1 » »	10 » »	55 » »	34 » »

On comparison with the corresponding figures relating to the autopsy series of pyloric stenosis cases without diarrhea hardly any essential differences are observable. In the entire region of the pyloric canal the ganglia investigated show the following ratios: A ganglia 3 per cent, B ganglia 9 per cent, C ganglia 54 per cent, and D ganglia 34 per cent.

The relation between the relative number of ganglia of each class and the duration of the disease is seen from the graph (fig. 37). There are four groups of duration of the disease:

Group	III,	duration of illness	21— 30	days	(5 cases,	13 sections)
»	IV,	» » »	31— 50	»	(6 »,	21 »)
»	V,	» » »	51—100	»	(5 »,	18 »)
»	VI,	» » »	114	»	(1 case,	4 »)

It is seen from the graph that the symptoms of pyloric stenosis have been of at least 3 weeks' duration in the cases belonging to this series. The patients who died at this stage had few A and B ganglia, only a small percentage, whereas the number of D ganglia

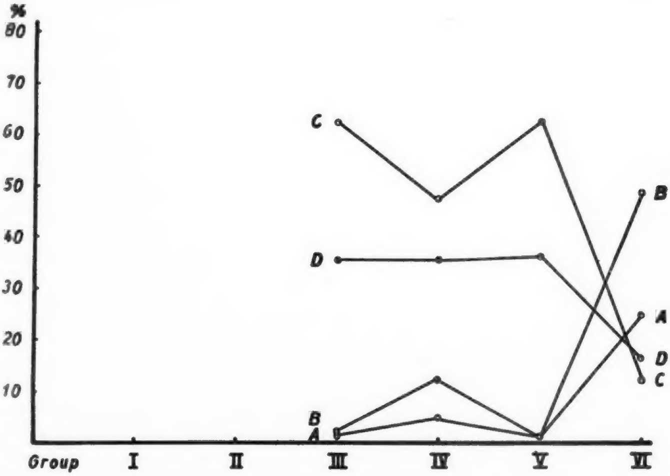


Fig. 37. — The abundance of ganglia of different classes in relation to the duration of illness in hypertrophic pyloric stenosis complicated with diarrhea, in the autopsy series. The illness has been reckoned from the first manifestation of the symptoms of pyloric stenosis.

was about 35 per cent and that of C ganglia about 60 per cent. After this a slight, slowly progressing recovery takes place. One of the infants is comparatively old, 5 months, and it constitutes group VI. In this case the combined number of good ganglia, A and B, is slightly over 70 per cent, that of C ganglia about 12 per cent and D ganglia about 16 per cent. With the exception of one case the number of D ganglia remains nearly constant even in this series throughout the time of observation, which is about 4 months. Conditions are thus quite similar to those in the autopsy series relating to pyloric stenosis without diarrhea.

Case L 3/46 is a fine example of distinctly increased nervous activity, side by side with which signs of regeneration are observable. The patient was admitted to hospital at the age of one month. He had vomited since birth. During the entire time in hospital, 10 days, projectile vomiting diarrhea and occurred. At autopsy the stomach was found to be narrower and longer than normal. Its walls were of more than normal thickness. The mucous membrane was hyperemic everywhere. The pylorus was clearly narrower than normal and the pyloric ring was considerably thickened.

Histologically a great number of nerve cells of types I and II were observed in the sections and the ganglia in which such cells

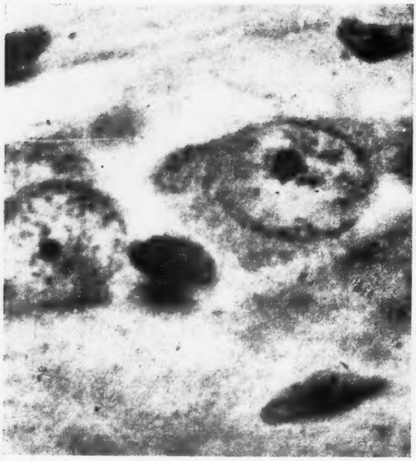


Fig. 38.

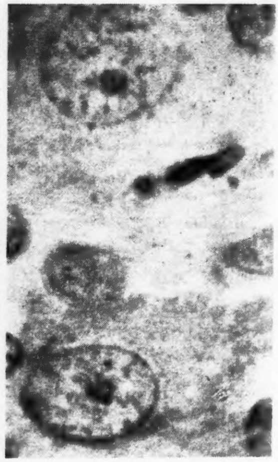


Fig. 39.

Figs. 38 and 39. — Nerve cells of type I in an A ganglion from the pyloric stenosis + diarrhea series, case L 3/46, P.c.mi. Gallocyanin staining at pH 1.7. — Magnification × 1800 in fig. 38, × 1450 in fig. 39.

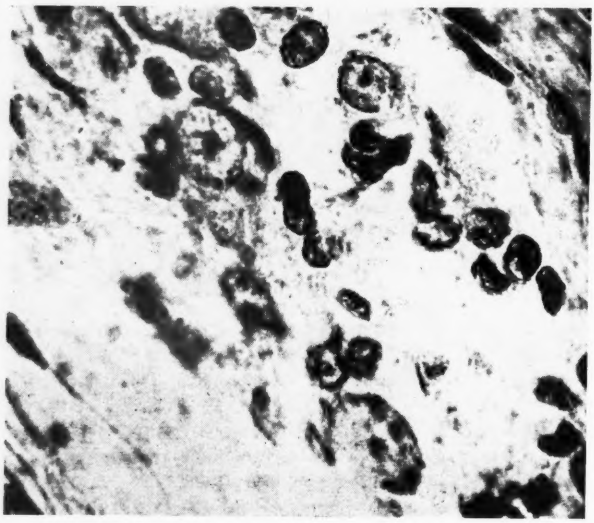


Fig. 40. — B ganglion from the pyloric stenosis + diarrhea series, case L 3/46, P.c.mi. Gallocyanin staining at pH 1.7. All visible nerve cells are intensely active nerve cells of type II. — Magnification × 980.

were found were thus in good condition. However, C and D ganglia were also present.

Figs. 38 and 39 present some fine nerve cells of type I from an A ganglion of case L 3/46, P.c.mi. The nuclear chromatin occurs in widely spaced, small accumulations in the nucleus, and the cytoplasm is uniformly stained. The nucleoli are very deeply stained, which may be due to the fact that the cells are in a state of regeneration.

Fig. 40 shows a B ganglion from the same section. The ganglion reveals that the nervous activity is increased. All the nerve cells visible in the picture are intensely active. The nucleotides are abundant in the periphery of the cytoplasms. These cells are consequently of type III.

The B ganglion in fig. 41 is a further example from the same section as the preceding figures. It has to be considered an intermediate form between the A and B ganglion, the acceleration of the nervous activity appearing to be relatively insignificant. At the upper end of the ganglion, displayed by the upper picture, there are only nerve cells of incipient type II; at the lower end, further, one nerve cell of type II and at the extreme lower edge possibly a nerve cell of type I. This ganglion, too, is probably in a stage of regeneration.

Fig. 42 shows a C ganglion of the same case, although from a different specimen and staining. This ganglion is of very large size and the number of satellite cells is greatly increased. Among the few nerve cells of the ganglion there are nerve cells of type II; moreover one nerve cell of type III is visible on the left, and obviously also residues of type IV.

Fig. 43, too, shows a B ganglion of case L 3/46, P.c.mi., with a considerable number of satellite cells and nerve cells of type II. The group of four nerve cells in the centre of the ganglion is reproduced at higher magnification in fig. 44. The cells appear to be of type II and incipient type II. The structure of the ganglion is indicative of its previous action as a center of strong nervous activity. This ganglion is probably in a stage of regeneration.

Even in this case the changes in the *nerve fibers* are similar to those in the biopsies. The nerve fiber bundles are hypertrophied and their arrangement is irregular. This is evident in fig. 45, from case 211/46, P.c.ma. The nerve fiber bundle is very strongly dispersed.

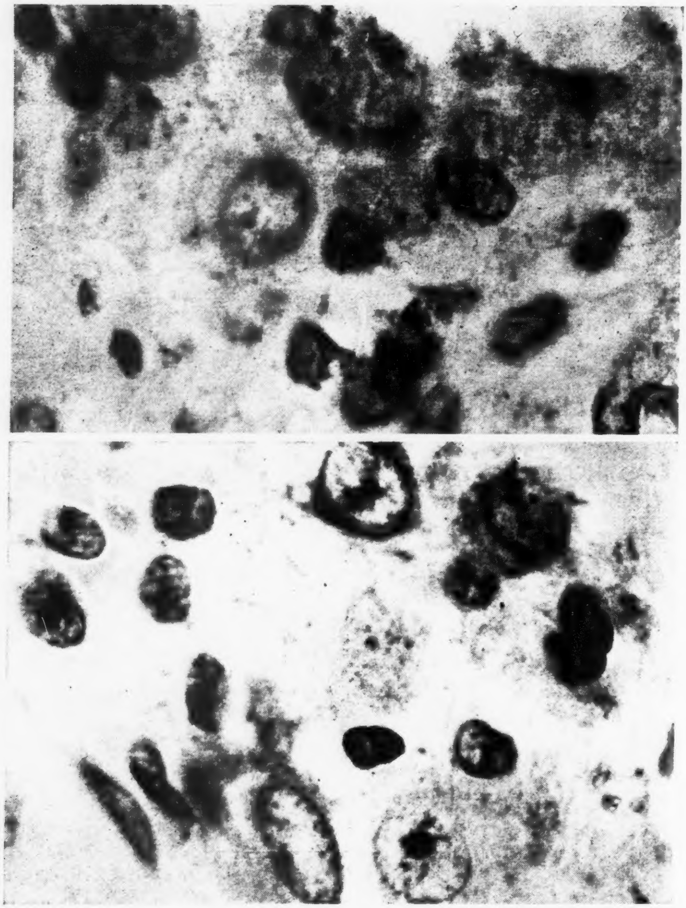


Fig. 41. — B ganglion from the same case and staining as that in fig. 40, sectioned through the middle. In the upper part of the ganglion, numerous nerve cells of incipient type II. Above in the lower part of the ganglion, a nerve cell of type II and below obviously a nerve cell of type I with a large nucleolus. Magnification × 1500.

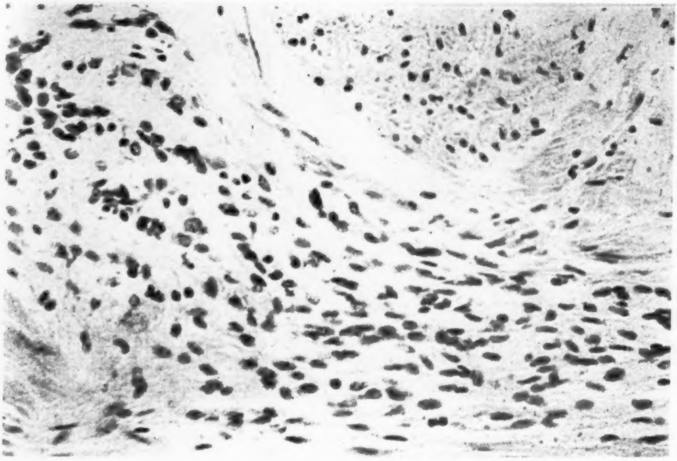


Fig. 42. — C ganglion from the pyloric stenosis + diarrhea series, case L 3/46, P.c.mi. Gallocyanin staining at pH 2.6. The large ganglion contains only a few cells, mainly of type II. In the lower left corner, a large, pale nerve cell of type III (arrow). The satellite cells are greatly increased in number. — Magnification × 300.

Fig. 43. — B ganglion from the pyloric stenosis + diarrhea autopsy series, case L 3/46, P.c.mi. Gallocyanin staining at pH 2.6. In addition to nerve cells of type II there are numerous cell ghosts which may be in a stage of regeneration. Strong nucleoli. Numerous satellite cells. — Magnification × 300.

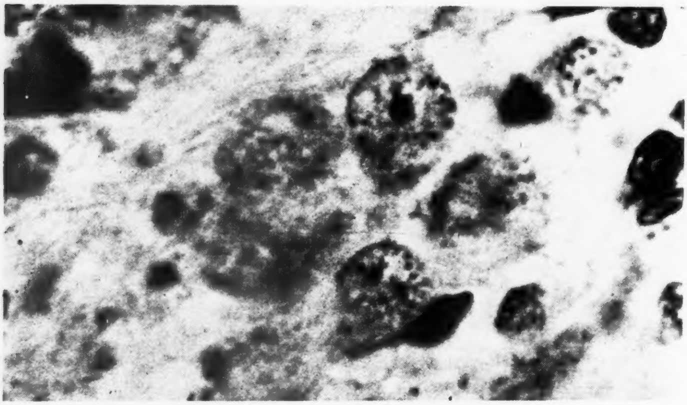


Fig. 44. — Higher magnification of the cell group in the centre of the ganglion in fig. 40. The nerve cells can be considered as being in a stage of regeneration. — Magnification × 1500.

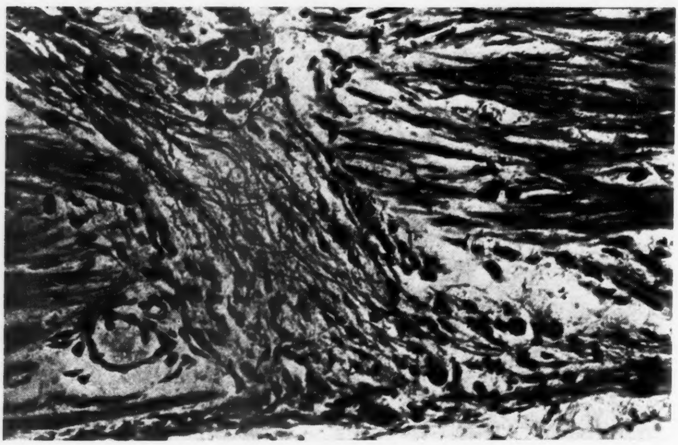


Fig. 45. — Strongly dispersed nerve fiber bundle from the pyloric stenosis + diarrhea series, case 214/46, P.c.ma. Bodian's stain. — Magnification × 300.

Leukocytes occur as in the preceding series, but the inflammation of the mucous membrane is more severe in degree.

5. DIARRHEA SERIES

The diarrhea series investigated consists of 8 cases, including 3 male and 5 female infants. Their ages range between 11 days and 3½ months. The duration of diarrhea had been from 4 days to 3 weeks. In addition to diarrhea there has been pneumonia in four cases, meningitis and encephalitis in two, and hydrocephalus in two cases, one of which, moreover, had a meningomyelocele. The cause of death was diarrhea in seven cases, one of them complicated by pneumonia. In one case purulent meningitis was the cause of death.

In the less severe cases of the diarrhea series hardly any degenerative changes in the nerve cells and ganglia are observable. The more severe cases are characterized by edema. The number of cells may frequently be remarkably low and the ganglia are apparently in poor condition. Although the ganglion frequently has a poor external appearance, its nerve cells nevertheless resemble type I or incipient type II.

The majority of the nerve cells are pycnotic. These cells often seem to have a more deeply stained nuclear membrane and nucleolus, thus resembling the nerve cells of type II in their external appearance. Their cytoplasm is deeply stained as a rule; consequently at least no deficiency in nucleotides can be observed in the cytoplasm of these cells. Tigrolytic nerve cells are rarely encountered; at least they are not generally observable with certainty.

In the diarrhea cases investigated no changes of similar character and of such progressivity have been noticed in the nerve cells as in hypertrophic pyloric stenosis, nor is any remarkable increase in the numbers of satellite cells observable. Therefore it does not seem possible that diarrhea would to any noteworthy degree mask the cell picture of hypertrophic pyloric stenosis in those cases where diarrhea occurs as a complication.

The Bodian staining in the diarrhea series was so unsuccessful that it is difficult to judge the state of the *nerve fibers*.

Diarrhea case L 123/47 was nearly 2 months of age on admission to hospital. The antecedent history revealed that there had been fever and occasional convulsions since the age of 2 weeks. Before admission there had been diarrhea and vomiting for two weeks. The patient died on the morning of admission. The autopsy diagnosis was purulent meningitis,

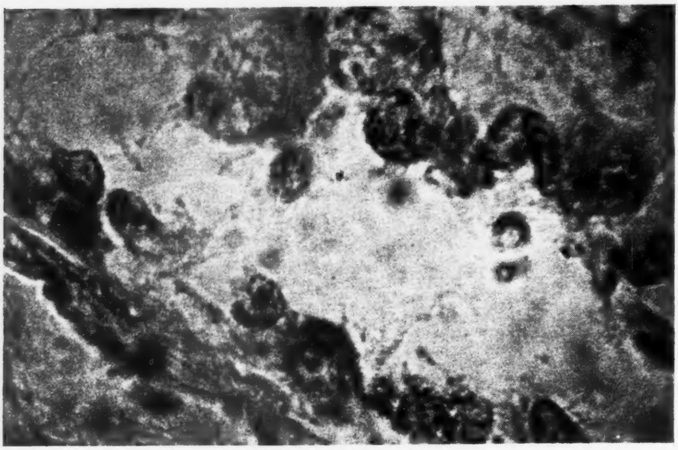


Fig. 46. — Ganglion from the diarrhea series, case L 123/47. Gallocyanin staining at pH 1.7. In the upper part, a nearly normal nerve cell. On the right and left, pycnotic nerve cells. Edema in the ganglion. Magnification × 1300.

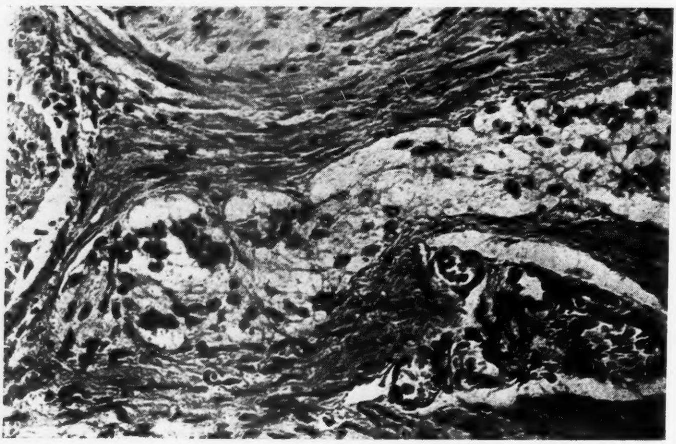


Fig. 47. — Ganglion from the diarrhea series, case 299/47, P.ventr. Gallocyanin staining at pH 3.4. Severe diarrhea. The majority of the nerve cells are pycnotic. Edema in the ganglion. — Magnification × 300.

internal hydrocephalus and encephalitis. The stomach had a normal appearance and its mucous membrane was light in color.

Fig. 46 shows an edematous ganglion of the case in question. On the right there is a pycnotic nerve cell with a large, deeply stained nucleolus and ample cytoplasm. The clearly visible nuclear membrane is puckered. In the lower part of the picture more pycnotic nerve cells are seen, and above a nearly normal nerve cell. The interstitial substance of the cells is badly decomposed in this ganglion.

Case 299/47 was 2 months old on admission and the diarrhea had persisted for one week. During four days the feces were green. The patient remained in hospital for slightly over 3 weeks, the diarrhea being alternately more and less severe, until ultimately the condition of the patient deteriorated and exitus ensued. The patient was then 3 months old and the illness had had a duration of about 1 month. At autopsy yellowish green stomach contents were found in the stomach and the mucous membrane of the stomach was hyperemic in places. Furthermore, paravertebral pneumonia was established.

Fig. 47 shows a ganglion from this severe diarrhea case. The nerve cells are pycnotic as a rule and edema is observable in the ganglion.

C. THE REGENERATION OF THE NERVE CELLS

The fact that infantile hypertrophic pyloric stenosis may be cured even without operation is evident from the improvement in the histologic picture of the ganglia in the cases of longer standing (figs. 38—40 and 43, 44). The graphs relating to the duration of the disease show that even one month after the manifestation of symptoms, indications of recovery begin to be visible in the nerve cells and ganglia. The number of C ganglia with far advanced changes begins to decrease and correspondingly that of the A and B ganglia in better condition is on the increase. This phenomenon can be observed in all the groups in which hypertrophic pyloric stenosis was present. According to Stöhr (1951) there are in the ganglia small, immature cells resembling nerve cells, with a minute cytoplasm and not yet differentiated into nerve cells. In case of need these cells can develop into active nerve cells. If we follow this concept of Stöhr's, the return of the activity of the ganglion

can, in part at least, be interpreted as due to the differentiation of these cells. The ganglion may have experienced extensive damage, of which the abundance of satellite cells is frequently a persisting indication. It is thus possible that in such an extensively changed ganglion recently developed nerve cells of type I occur, which would obviously render the ganglion at least partly fit for activity. The cells seen in figs. 23 and 30 of the biopsy series, for instance, are probably such young nerve cells. The cell in the centre of fig. 23, adjacent to an intensely active nerve cell of type II, is obviously a nerve cell of this kind in the process of development. The cells in the upper left part of fig. 30 can be similarly interpreted.

However, regeneration of the ganglion also takes place in another way. If the ganglion contains nerve cells in an exhausted state, which have not yet gone into necrosis, these will slowly recover their activity after the cause of the damage has been eliminated. Recovery obviously takes place in the same way as was observed by Hydén in his stimulation experiments (cf. fig.3).

If we now consider case L 3/46, our attention is attracted in the histologic survey, in the first place, by four facts:

— the section contains ganglia in good and poor condition side by side,
— ganglia rich in satellite cells may contain fine specimens of nerve cells of type I,
— the nerve cells of type I frequently have a nucleolus more deeply stained than usual,
— the ganglia contain a great amount of indefinable cell residues.

The disease had a duration of 41 days in this case. For this reason we are probably justified in interpreting the parallel occurrence of ganglia in relatively good condition on the one hand and of ganglia displaying far advanced changes on the other hand by assuming that the ganglia in better condition were in the process of regeneration, and had already been restored in part. The strong nucleolus of the nerve cells of type I (figs. 38 and 39) can be considered one indication of this. The nucleolar apparatus of the cells seems to have been vigorously active in the production of cytoplasmic proteins, and after equilibrium has been attained the apparatus is undergoing a retrograde development, the nucleolus still presenting a staining reaction of more than normal strength.

It would indeed be difficult to conceive of nerve cells with deeply stained nucleoli as nerve cells of type I in their stage of activation, since at the onset of hyperfunction of the nucleolar apparatus nucleic acids appear in abundant amount in the nuclear membrane simultaneously with the increase in stainability of the nucleolus, a phase which already coincides with the transition of the cell into a nerve cell of type II (cf. figs. 3b and 3d). Thus probably the nerve cell of type I with deeply stained nucleolus is to be considered a regenerated nerve cell.

The B ganglion shown in fig. 41 should possibly also be regarded as a ganglion in the process of regeneration. The nerve cells in the upper picture are already returning to normal and some may be referred to the incipient type II. They would represent the stage of recovery in which the amount of nucleotides in the nucleus is on the increase. In the lower picture nerve cells of type II and a nerve cell of type I with a deeply stained nucleolus are seen, the vigorous activity of which is obviously already on the decline. This picture reveals that the nucleolus is possibly the last to regain its normal shape. The ganglion also contains a great number of satellite cells as relics of its vigorous activity.

Fig. 43, from this same case, also shows a large B ganglion rich in satellite cells, with a great number of nerve cells which can probably also be interpreted as cells in process of regeneration. Even in these the large nucleolus attracts particular attention. Fig. 44 gives a higher magnification of the group of four nerve cells in the centre of this ganglion. The cells are most nearly of incipient type II and thus returning to their normal state. The structure of the ganglion indicates that it has been the center of increased nervous activity, and it is likely that these signs, the large size of the ganglion and its satellite cells, would not have disappeared at the regeneration of the ganglion.

As an illustrative example of recovery we may also cite case 164/45. The patient was 5 months old at death. The clinical symptoms of hypertrophic pyloric stenosis had already ceased at this stage. Histologically a great number of ganglia in good condition and very rich in cells, with a considerable number of satellite cells, were observed (fig. 48). The ganglion cells, which were of different sizes, were fairly uniformly distributed throughout the ganglion. The occurrence of the different classes of ganglia in the pyloric

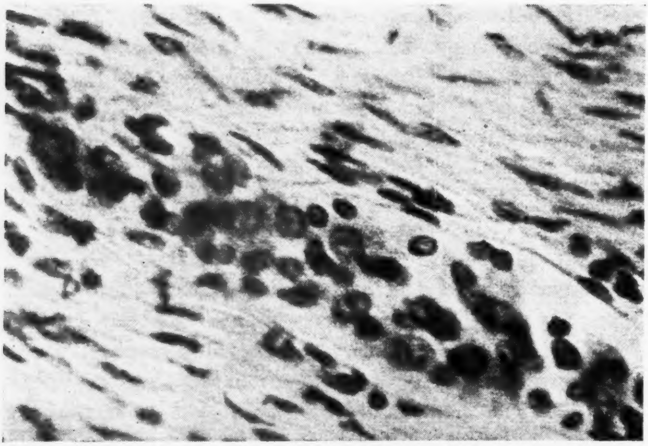


Fig. 48. — Regenerated A ganglion from the pyloric stenosis + diarrhea series, case 164/45. Gallocyanin staining at pH 3.6. The ganglion is from a 5-month-old patient who had recovered from pyloric stenosis but had later died of hemorrhagic gastritis. The ganglion contains numerous fine nerve cells of type I. The satellite cells are also fairly numerous. — Magnification × 600.

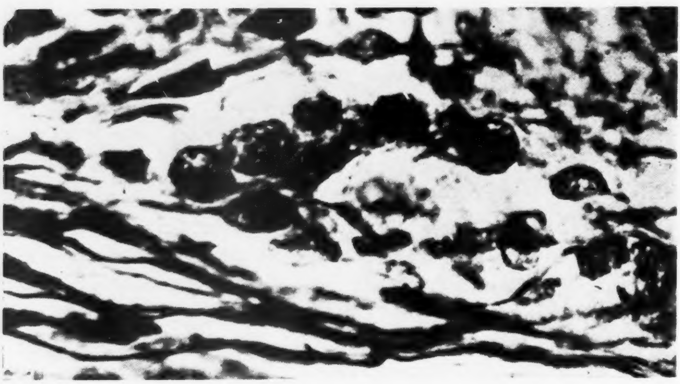


Fig. 49. — Regular nerve fiber bundle departing from an A ganglion of a 5 month-old case (164/45) which has recovered from pyloric stenosis. Bodian's stain. — Magnification × 1000.

region was as follows: A ganglia 24 per cent, B ganglia 48 per cent, C ganglia 12 per cent, D ganglia 16 per cent.

Leukocytes occurred in some amount perivascularly, and slight inflammation was observed in the mucous membrane. The nerve

fibers generally travel as regular bundles in the manner of normal nerve fibers. Fig. 49 shows an A ganglion of this case with its nerve fibers.

The case in question, 164/45, had had hypertrophic pyloric stenosis, but had recovered from it and later died from hemorrhagic gastritis.

The patient, a female infant, was a first child. The birth weight was 3200 g. She had begun to vomit at slightly over one month of age. On admission to hospital the weight was 3280 g and the age 41 days. Treatment was conservative. At four months of age the vomiting was negligible and the weight was increasing. During the last two weeks of life no vomiting occurred at all, with the exception of the last two days.

At the age of 2½ months, furthermore, inflammation in the right gluteal region was observed, which was later established roentgenologically as osteomyelitis of the right ilium. At the time of death the weight was 3130 g.

The autopsy revealed atrophy, the heart was dilated, the surface of the pericardium was not perfectly glossy and the pericardium contained a small amount of turbid fluid. On the boundary between the right auricle and ventricle a white, bloodless area of about 3 by 3 cm (embolus) was found. The cardiac muscle was glossy in appearance. In the endocardium nothing of significance. Persistent Botallo's duct. In the lungs no pneumonic foci. The pylorus was long and thick, its contents hemorrhagic. Small hemorrhages in the mucous membrane. Parenchymal degeneration in the liver. Spleen rather large, flaccid and of indefinite structure. Kidneys and adrenals: nothing of significance.

D. COMPARISON OF THE NERVE CELL PICTURES OBTAINED WITH GALLOCYANIN STAINING AND WITH THE U.V. ABSORPTION METHOD

When the present investigation was nearly completed, the equipment for ultraviolet absorption photography at the Institute of Forensic Medicine of the University of Helsinki was put into service and the opportunity was taken to photograph the nerve cells of the present series in ultra-violet light and to make a comparison between such photographs and the microscopic pictures obtained with the aid of gallocyanin staining. As the object of such a comparative study a nerve cell of type I from case 164/45, diarrhea + pyloric stenosis, and a tigrolytic nerve cell of type III from the biopsy case 3072/48 were chosen.

In both cases the different types of pictures were made from one and the same section. The section, which was covered with glycerol, was first photographed according to the U.V. absorption method at the maximum absorption wavelength of the nucleic acids, 2650 Å, whereupon the section was stained with gallocyanin at pH 1.7 and microphotographed. Figs. 50 and 52 show the U.V. photographs of these cells and figs. 51 and 53 the corresponding gallocyanin stainings.

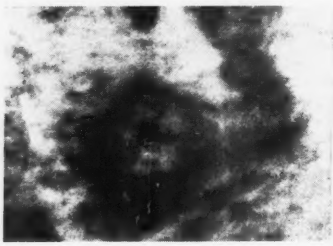


Fig. 50.

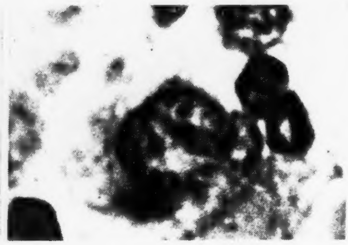


Fig. 51.

Figs. 50 and 51. — The figures represent nerve cells of type I from case no. 164/40. — Fig. 50 is a U.V. absorption photograph at 2650. Å. The cytoplasm absorbs strongly, particularly in the vicinity of the nuclear membrane, there are some strongly absorbing areas in the nucleus, and the nucleolus is of small size. The absorption is due to the nucleic acids and in part to the proteins. — Fig. 51 is obtained from the same nerve cell with gallocyanin staining at pH 1.7. The cytoplasm is rather weakly stained elsewhere, except at the lower edge of the cell, where a dark region is seen. The nuclear chromatin is agglomerated and the nucleolus is of small size. The staining is due to the nucleic acids. That is why the dark regions are not as wide and dense as in the U.V. absorption photograph. — Magnification about × 1600. Numerical aperture 1.25 corr.f. 2536 Å.

In the nerve cell of type I, which was considered normal on the strength of the gallocyanin staining, the U.V. absorption photograph (fig. 50) shows a region of strong absorption throughout in the cytoplasm, particularly in the vicinity of the nuclear membrane. In the nucleus there are accumulations of matter which display a stronger absorption than their surroundings, and the nucleolus is of fairly small size. Although the photograph was made at the wavelength of maximum absorption of the nucleic acids, the light rays are also absorbed to a certain degree by the proteins. It is thus possible to conclude from the picture that in the cytoplasm, and particularly close to the nuclear membrane, the concentration

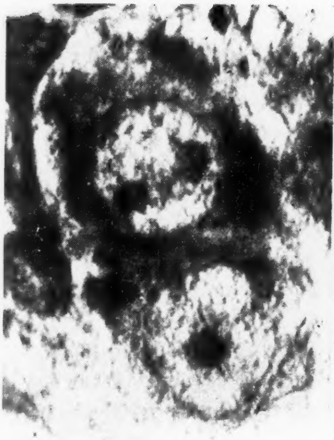


Fig. 52.

Fig. 53.

Figs. 52 and 53. — The figures represent nerve cells of type III from the biopsy case 3072/48. — Fig. 52 is a U.V. absorption photograph at 2650 Å. The nuclei of the nerve cells are large in size and display a fairly low absorption. The nucleoli are large and strongly absorbing. One of the nerve cells has two large nucleoli as an indication of strong activity. The cytoplasm of this nerve cell displays a low absorption in places, although the absorption is still very strong at some points. The nerve cell has to be considered an incipient stage of a nerve cell of type III. — Fig. 53 shows the same nerve cells stained with gallocyanin at pH 1.7. The nucleoli are deeply stained, there are conglomerations of chromatin in the nucleus, and in the partially tigrolytic cytoplasm some more deeply stained regions still remain. The stained areas are caused by nucleotides. The U.V. absorption photograph and the gallocyanin staining are in good agreement. — Magnification about × 1600. Numerical aperture 1.25 corr.f. 2536 Å.

of both nucleic acids and proteins is considerable and that these substances occur in aggregates in the nucleus. The nucleolus is not increased in size. All these are features characteristic of the normal nerve cell.

In the corresponding gallocyanin staining (fig. 51) a typical normal nerve cell is seen, with a fairly low nucleic acid content of the cytoplasm except for a region close to the nuclear membrane, where the concentration is higher. Moreover, the nucleic acids in the cytoplasm seem to be granulated. The nuclear chromatin appears in large accumulations in the nucleus, and the nucleolus is small.

In the tigrolytic nerve cells of type III distinct signs of extremely

accelerated activity are seen in the U.V. absorption photograph (fig. 52): the nuclei are swollen (compare with the preceding nerve cell, which is reproduced at the same magnification), the nucleoli are large and show strong absorption. The cell nucleus is situated at the periphery, and in the cytoplasm close to the nuclear membrane, particularly in the peripheral part of the cytoplasm, there are regions of strong absorption in which the content of nucleic acids is high. However, tigrolysis is distinctly observable in the cell nuclei and also in the cytoplasm of the upper cell. The nerve cells thus have to be considered nerve cells of type III in an early stage, the tigrolysis having only recently set in.

In the corresponding gallocyanin picture (fig. 53) all the above-mentioned phenomena are similarly seen; the swelling of the nucleus, the large, deeply stained nucleoli, dark areas in the peripheral region of the cytoplasm, and tigrolysis.

The above-mentioned pictures show that the U.V. absorption picture and the picture obtained by gallocyanin staining are in entire agreement with each other. There only exists a difference insofar as the dark, absorbing areas in the U.V. photographs are somewhat larger in extent and of higher density than the corresponding stained areas in the gallocyanin. In the latter, moreover, these stainings are granulated. This difference is quite understandable, since in the gallocyanin staining (at pH 1.7) only the nucleic acids are stained, the pictures thus showing the localization of the nucleotides in the nerve cell. In the U.V. absorption photographs, on the other hand, proteins are also visible in addition to nucleic acids. A comparison of the pictures of tigrolytic cells reveals that in those places where no nucleic acids occur, proteins are also absent, which is in accordance with Hydén's experimental results. This observation was also made in the course of the work when the staining was carried out at a higher pH (3.4), part of the proteins also being stained (fig. 12). All this is immediately evident from the pictures. In the normal nerve cell or in a nerve cell with increased activity (type II), however, conditions may be different. In a nerve cell in its normal state the absorption in the cytoplasm is high enough in the initial stage to mask, at ocular assessment, the increase of the nucleic acids in the cytoplasm close to the nuclear membrane, which is due to the increased activity. For this reason it will scarcely be possible to make a positive distinction between

nerve cells of type I and those of incipient type II from the U.V. absorption photograph merely on the basis of the picture, without recourse to comparative determinations of the absorption intensity. With gallocyanin staining, on the other hand, this distinction is directly possible, as was seen above in the classification of the nerve cells. It is particularly by its simplicity and rapidity that the gallocyanin method has proved itself a valuable asset in the present investigation, which has involved the examination of a comparatively large material, including thousands of ganglia, with the object of observing the changes in the nerve cells. The classification of nerve cells and ganglia is possible merely on the basis of a microscopic study of the staining. The experiment of U.V. absorption photography described above provides convincing proof of the fact that the deductions drawn from the gallocyanin stainings with regard to the different states of activity of the nerve cells do indeed comply with reality.

VII. DISCUSSION

The aim of the present investigation has been to gain insight into the changes observable in the nerve cells of the myenteric plexus in the pyloric canal of infants suffering from hypertrophic pyloric stenosis. On the assumption that the changes are related with the metabolism of the nerve cells, which in its turn is associated with the occurrence of nucleic acids in the cells (Hydén 1943a), the well-known and thoroughly investigated gallocyanin—chrome alum staining method (Einarson 1932, 1947, 1951; Einarson and Bentsen 1939) has been chosen in order to make these changes visible by means of histologic staining. With this method it was possible to make observations concerning the occurrence of nucleic acids in the nerve cells and thus also concerning their protein metabolism.

In order to eliminate any possible postmortem changes, the conclusions have mainly been drawn from the biopsy series, although autopsy material has also been used to supplement the biopsy findings.

In the myenteric plexus of the pylorus extensive changes have been observed in the nerve cells. On the basis of these changes the nerve cells could be divided into four types. In the nerve cell of type I considerable basophily is observable both in the nucleus and in the cytoplasm, which is referable either to the content of nucleic acids or to this in conjunction with part of the proteins, depending on the pH of the staining solution employed. This nerve cell type is that of a normal cell in a state of rest. In type II strong basophily occurs in the system nucleolus—nuclear membrane, which indicates that the protein-producing mechanism is highly active (Hydén 1943a). Cell type III is a tigrolytic cell, indicative of a low amount of nucleic acids and a state of exhaustion of the cell. Cell type IV is a necrobiotic, disappearing cell.

The changes in the nerve cells observed in this investigation are equivalent to the changes produced by means of various stimuli. These changes have been studied, employing quantitative methods for investigating nucleic acids and proteins: ultraviolet absorption spectrography and roentgen-microradiography (Hydén 1943a, 1944, 1945, 1952; Hamberger and Hydén 1945, 1949a, 1949b; Floberg et al. 1949; Hochberg and Hydén 1949; Brattgård 1952). This observation immediately suggests the thought that the primary cause of the changes observable in the myenteric plexus in infantile pyloric stenosis might be some stimulus.

In the course of the disease a change in the state of the nerve cell lesions has been noticed. In the earliest material under investigation the symptoms had prevailed for 9—10 days. Even at this time the damage to the nerve cells was found already to have achieved its maximum. Therefore it seems evident that the clinical symptoms of stenosis commence when the changes in the nerve cells have attained a certain degree.

When clinical symptoms have prevailed for about 3—4 weeks, signs of recovery can be observed in the nervous tissue. This requires that the pathologic stimulus which has produced the nervous damage — if we consider stimulation to be the cause of these phenomena — has subsided or at least lessened and that regeneration has thus been enabled to set in.

Regeneration of the nerve cells obviously takes place upon cessation of the pathologic stimulus, the tigrolytic cells or those cells which have experienced less severe changes being then slowly restored to their initial state, which is the way in which Hamberger and Hydén (1945), for instance, in their experimental investigations, have shown this restitution process to occur. Since, moreover, in autonomic ganglia cells resembling neuroblasts are also always seen, it appears obvious that these cells may differentiate into active nerve cells, which then replace such cells as have lost their normal activity (Stöhr 1949, 1951). However, the ganglia from which the nerve cells have vanished (D ganglia) probably cannot be regenerated, as is borne out by the fact that the number of such ganglia remains nearly constant throughout the period of observation. In the beginning, at least, they thus remain in the tissue as ganglion residues made up of satellite cells.

What, then, may be the pathologic stimulus that has been

able to produce such damage to the nervous system as was observed in the present investigation? It is possible that the initial stimulation is due to inflammation, as leukocytes have been observed in considerable numbers in the ganglia in the beginning of the disease. It obviously is particularly easy for the cause of the inflammation to reach the myenteric plexus of the sphincter, which is in close connection with the mucosa, since the circular muscle layer is absent at the pyloroduodenal junction (Horton 1928, Belding and Kernohan 1953). Furthermore the opening muscle, which is formed from the longitudinal muscle layer, is still rather weak at this age, since it is not yet fully developed at birth (Horton 1928). Segments of longitudinal muscle have been found to be absent both in infants who have hypertrophic pyloric stenosis and in normal infants (Belding and Kernohan 1953). It is only fully developed during the first year of life (Welch 1921—22).

The inflammation findings are in accord with Holsti's (1931) investigations. In pyloric affections of adults which simulated ulcer and which had symptoms of pyloric stenosis, he has found neutrophil granulocytes in the myenteric plexus. In these cases he has also observed changes in the nerve cells, such as tigrolysis, karyolysis and pycnosis.

Thus, from the patho-anatomic point of view, inflammation would seem to play a major part in the genesis of pyloric stenosis, a fact which Räihä and Ylppö (1939) have stressed on the basis of clinical observations.

As a working hypothesis it is thus possible on the strength of the patho-anatomic findings to make the following statement: The cause of inflammation may be neurotropic factors, which, moreover, may be selective. When they affect the nervous system, they cause a pathologic increase of the nervous function. Simultaneously phagocytosis by the leukocytes occurs in the region of inflammation. Through the influence of these phenomena the nervous system of the opening mechanism is gradually deranged and the opening muscle is no longer able to function in the normal way. This circumstance in its turn increases the motility of stomach and pylorus. When the increased motility of the stomach tends to open the sphincter by means of the already partially degenerated nervous system, it is only able to open it incompletely. This results in a state of tension of the sphincter. It manifests itself in a kind of tremor in

the sphincter and in an intramuscular tension of the entire pylorus. In its capacity of motor stimulus, this trembling motion in combination with the increased motility again increases both the degeneration of the nervous system and the functional hypertrophy in the muscle as well as in the nervous tissue. Consequently the initial stimulus originating from inflammation and, on the other hand, the motor stimulus due to the movements of stomach and pylorus together produce the histologic picture of the nervous tissue seen in pyloric stenosis. Their influence seems to be fairly rapid, since the resultant changes are already fully developed in the earliest cases that could be secured for investigation.

After this stage the nervous damage does not essentially change. Not until about one month after the onset of the disease are the first signs of recovery noticeable. This tends to indicate that the initial stimulus cannot have been of very long duration. This is supported by the observation that leukocytes have been observed in the myenteric plexus only for about three weeks after the onset of symptoms. About two weeks later regeneration can be observed histologically, which tallies with the phenomena recorded by Hamberger and Hydén (1945), in their stimulation experiments, upon cessation of the stimulus.

Recovery proceeds rather slowly, as damage is still observable in the nervous tissue after 3—4 months' duration of the disease. This is probably due to the fact that the pathologic motor stimulus is still present even though the stimulus caused by inflammation has ceased; this is a result of the fact that the narrow lumen of the hypertrophied pyloric canal and the still incompletely recovered opening mechanism continue to obstruct the passage of food.

The nervous damage observed in pyloric stenosis was severe as a rule. Necrobiotic nerve cells and the destruction of entire ganglia could be observed in great profusion. Hamberger and Hydén (1945) have shown experimentally that after a pathologic stimulus of such violence as to cause necrosis of part of the nerve cells, regeneration has taken about 2 months and even at that time restitution has not been complete. Accordingly in pyloric stenosis in patients of 3—4 months of age a considerable, though not yet complete, recovery should be observable. This could indeed be noticed in the investigations and is also in accord with the clinical observations.

Following this line of reasoning, the interpretation of the recovery of operated patients is that the motor stimulus ceases on section of the nerve connections, and the nerve cells find an opportunity to regenerate. The inflammation also has time to subside before new nerve connections are formed. According to Wollstein (1922) the formation of these connections requires between 2 months and 2 years.

The ultimate reason why some infants have pyloric stenosis while others are free of it may be found in their constitution. It has been found that persons who have had pyloric stenosis as infants have as adults a higher than normal incidence of gastrojejunitis or gastrojejunal ulcer (Belding and Kernohan 1953). Moreover, general lability of the nervous system is evident in these persons, and even in their relatives in a higher degree than in other individuals (Bendix and Necheles 1947, Salmi 1941, Nieminen 1952).

Finally the question may be raised why diarrhea does not cause changes in the myenteric plexus similar to those observed in pyloric stenosis. We may answer this by saying that probably the main reason is the absence of a neurotropic inflammation stimulus in diarrhea, since no leukocytes have been encountered in the myenteric plexus in diarrhea.

VIII. SUMMARY

In this investigation aimed at determining the histopathologic changes in the myenteric plexus of the pylorus observable in hypertrophic pyloric stenosis of infants the material consisted of 56 cases. 18 further cases were studied as a control series. Both autopsy and biopsy cases are included in the series. In the autopsy series the sections were taken from four points of the pyloric canal: from the ventral and dorsal walls and from the walls of the lesser and greater curvature. The observations concerned the changes in nerve cells, ganglia and nerve fibers and the occurrence of leukocytes. Altogether more than 2000 ganglia were investigated. The sections, mainly fixed in formalin, were stained for the nerve cell and ganglion investigations with Einarson's gallocyanin—chrome alum stain, using solutions of several different pH values. The nerve fibers were studied from silver stainings according to Bodian's method and the leukocytes from general stainings made with hematoxylin-eosin.

The hypertrophic pyloric stenosis material falls into three series: the biopsy series, the autopsy series without diarrhea, and the autopsy series of cases with diarrhea in addition to hypertrophic pyloric stenosis. In all series similar changes in the nervous system were observed.

The control material falls into two series: the series free of diseases of the digestive system, and the diarrhea series. In the nerve cells and ganglia of the diarrhea series changes were observable in comparison with the control series free of diarrhea. However, these changes were somewhat different from those in the hypertrophic pyloric stenosis series. The pycnotic cells were characteristic of the diarrhea series, as well as the discontinuity of the tissue between the cells. It was found, however, that these changes could not mask the changes in the nerve cells caused by hypertrophic pyloric stenosis.

The principal observations relating to changes in the myenteric plexus of the pylorus and the conclusions which have been drawn from them may be summarized as follows:

1. Nerve cells and ganglia of different types and degrees were observed.

2. The nerve cells were divided into four types according to the observed morphologic changes:

Type I: A nerve cell in which the nuclear chromatin is distributed in widely separated masses uniformly over the nucleus, and Nissl substance is distributed in fine granules all over the cytoplasm. Type I has been regarded as a normal nerve cell.

Type II: A nerve cell with a deeply stained nucleolus-nuclear membrane system, which according to Hydén indicates a nerve cell in a state of intense activity.

Type III: A tigrolytic nerve cell, which can be considered in a state of exhaustion according to Hydén.

Type IV: A necrobiotic nerve cell with vacuolization in the cytoplasm and disintegration in the nucleus.

3. The ganglia were divided into four classes according to the nerve cell types occurring in them:

A ganglion: A ganglion in which the nerve cells of type I are predominant. This type was considered a normal ganglion.

B ganglion: A ganglion characterized by nerve cells of type II. In addition to these, nerve cells of types I and III also occur. The ganglion is thus in a state of hyperfunction.

C ganglion: A ganglion in which nerve cells of types III and IV are predominant. In addition to these nerve cells of types I and II may also occur. The number of nerve cells is generally diminished and that of the satellite cells is increased. The ganglion is in a state of exhaustion.

D ganglion: A ganglion containing satellite cells. The nerve cells are absent.

4. The damage described above was found already to have attained its maximum when the illness, as counted from the onset of projectile vomiting, had lasted 10 days. During the next few weeks there were no noteworthy changes in the lesion, but after about 1 months' duration of the disease a slight recovery begins to be discernible. When the changes are at their maximum, scarcely any A and B ganglia occur at all, the C and D ganglia being dominant.

At the commencement of recovery, A and B ganglia appear, and the C ganglia diminish at the same time. The number of D ganglia remains almost constant throughout the period under investigation. The restitution of the ganglia was still incomplete even after 5 months had elapsed since the onset of the disease, although the clinical symptoms had disappeared. The series still contained B and C ganglia.

5. The regeneration of the ganglia seems to take place in two separate ways:

— Cells held in reserve in the ganglion differentiate to constitute nerve cells.
— Tigrolytic cells in a state of exhaustion are restored to activity.

From the phase of regeneration satellite cells and cell shadows are left in the regenerated ganglia; moreover the ganglia are frequently of large size.

6. The nerve fiber bundles are hypertrophied and dilated; further they present a considerable disorder. Thickenings are seen in the individual fibers.

7. The observations concerning leukocytes in the biopsy series were that in the patients who had been ill for the shortest time these occurred in considerable numbers perivascularly, and in rather large numbers intraganglionally. With increasing duration of the disease the leukocytes decrease in number and are only rarely encountered when the disease has persisted for more than one month. From the material of the pyloric stenosis autopsy series no conclusions can be drawn with regard to inflammation cells, as other diseases have been simultaneously present.

8. It seems obvious that the damage to the nervous system is caused by a pathologic stimulus. This may be a neurotropic inflammation factor of some kind which enters the myenteric plexus of the sphincter. This results in increased nervous activity and in the simultaneous occurrence of phagocytosis by the leukocytes in the inflamed region. To this is added the influence of the increased motility of the sphincteral musculature. These phenomena result in changes in the nerve cells and derangement of the nerves of the opening system which makes the opening muscle unable to perform its function normally.

9. The clinical symptoms are a consequence of the damage to the nervous system which has been described above. When the stimulation caused by the inflammation subsides, a gradual regeneration of the nerve cells and disappearance of the clinical symptoms sets in. The regeneration takes place at rather a slow rate, since damage is still observable in the nervous tissue 3—5 months after the onset of the disease. Recovery is retarded by the circumstance that after cessation of the inflammation stimulus the pathologic motor stimulus still partly persists.

IX. ZUSAMMENFASSUNG

Das Material der vorliegenden Arbeit, welche als Ziel die Feststellung von histopathologischen Veränderungen im myenterischen Plexus bei hypertrophischer Pylorusstenose von Säuglingen hat, besteht aus 56 Fällen. Als Kontrollmaterial wurden weitere 18 Fälle untersucht. In der Autopsiereihe wurden von vier verschiedenen Stellen des Pyloruskanals Schnitte entnommen von der Ventral- und der Dorsalwand sowie von den Wänden der kleinen und der grossen Kurvatur. Die Beobachtungen bezogen sich auf die Veränderungen in Nervenzellen, Ganglien und Nervenfasern sowie auf das Vorkommen von Leukozyten. Insgesamt wurden mehr als 2000 Ganglien untersucht. Die zum grössten Teil in Formalin fixierten Schnitte wurden für das Studium der Nervenzellen und Ganglien mit Einarsons Gallocyanin—Chromalaun—Färbemittel gefärbt, wobei Lösungen von verschiedenem pH-Wert zur Anwendung kamen. Die Nervenfasern wurden an Hand von Silberfärbungen nach Bodian und die Leukozyten an Hand von Allgemeinfärbungen mit Hämatoxylin-Eosin untersucht.

Das Material in Bezug auf hypertrophische Pylorusstenose zerfällt in drei Reihen: die Biopsiereihe, die Autopsiereihe ohne Durchfall und die Autopsiereihe mit Durchfall neben hypertrophischer Pylorusstenose. In allen Reihen waren ähnliche Veränderungen des Nervensystems zu beobachten.

Das Kontrollmaterial zerfällt in zwei Reihen: die Reihe ohne Erkrankungen des Verdauungssystems sowie die Durchfallreihe. In den Nervenzellen und Ganglien der Durchfallreihe waren Veränderungen im Vergleich mit der Kontrollreihe ohne Durchfall wahrnehmbar. Diese Veränderungen waren jedoch einigermassen verschieden von denen in der Reihe der hypertrophischen Pylorusstenosefälle. Kennzeichnend für die Durchfallreihe waren pyknotische Zellen sowie ein unzusammenhängendes Gewebe zwischen

den Zellen. Es zeigte sich indessen, dass diese Veränderungen nicht imstande waren, diejenigen Veränderungen in den Nervenzellen zu verdecken, die durch hypertrophische Pylorusstenose herbeigeführt sind.

Die bedeutsamsten Beobachtungen in bezug auf die Veränderungen im myenterischen Plexus des Pförtners und die daraus gezogenen Schlüsse können folgendermassen zusammengefasst werden:

1. Es wurden Nervenzellen und Ganglien verschiedener Typen festgestellt.

2. Die Nervenzellen wurden nach den in ihnen beobachteten morphologischen Veränderungen in vier Typen eingeteilt:

Typ I: Nervenzelle, in der das Chromatin in weit auseinanderliegenden Massen gleichmässig über den Zellkern und der Nisslschen Schollen in kleinen Körnchen über das gesamte Zytoplasma verteilt sind. Typ I ist als Typ der normalen Nervenzelle angesehen worden.

Typ II: Nervenzelle mit stark gefärbten Nukleolus und Kernmembran, nach Hydén eine Nervenzelle in einem Stadium intensiver Aktivität kennzeichnend.

Typ III: Tigrolytische Nervenzelle, nach Hydén als Erschöpfungszustand deutbar.

Typ IV: Nekrobiotische Nervenzelle, Vakuolisation im Zytoplasma und Auflösung im Zellkern aufweisend.

3. Die Ganglien wurden nach den in ihnen auftretenden Nervenzelltypen in vier Klassen eingeteilt:

A-Ganglion: Ein Ganglion, in welchem Nervenzellen vom Typ I vorherrschend sind. Dieser Typ wurde als normales Ganglion angesehen.

B-Ganglion: Ein Ganglion, das durch Nervenzellen vom Typ II gekennzeichnet ist. Ausser diesen kommen auch Nervenzellen vom Typ I und III vor. Das Ganglion befindet sich somit in einem Zustand der Hyperfunktion.

C-Ganglion: Ein Ganglion, in welchem Nervenzellen der Typen III und IV vorherrschend sind. Ausser diesen können auch Nervenzellen von Typ I und II vorkommen. Die Zahl der Nervenzellen ist im allgemeinen vermindert und die der Satellit-

zellen erhöht. Das Ganglion steht in einem Stadium der Erschöpfung.

D-Ganglion: Ein Ganglion, welches Satellitzellen enthält. Die Nervenzellen fehlen.

4. Es wurde festgestellt, dass die oben beschriebenen Zerstörungen ihr Maximum bereits erreicht hatten, wenn die Krankheit 10 Tagen bestand, gerechnet vom Beginn des Sturzerbrechens. Während der nächsten Wochen ereigneten sich keine bemerkenswerten Änderungen im Umfang der Zerstörung, aber nach einer Krankheitdauer von etwa einem Monat ist eine leichte Besserung wahrnehmbar. Wenn die Veränderungen ihr Maximum aufweisen, kommen fast überhaupt keine A- und B-Ganglien vor, während die C- und D-Ganglien vorherrschen. Beim Beginn der Erholung erscheinen A- und B-Ganglien und zugleich nimmt die Zahl der C-Ganglien ab. Die Zahl der D-Ganglien hält sich die ganze Untersuchungszeit hindurch nahezu konstant. Die Erholung der Ganglien war noch unvollständig, nach 5 Monaten ab Krankheitsbeginn, obwohl die klinischen Symptome verschwunden waren. Das Material enthielt noch B- und C-Ganglien.

5. Die Regeneration der Ganglien scheint auf zwei verschiedenen Wegen stattzufinden:

- Im Ganglion als Reserve vorhandene Zellen differenzieren sich zu Nervenzellen.
- Tigrolytische Zellen im Erschöpfungszustand werden zu aktiven Zellen wiederhergestellt.

Von der Regenerationsphase bleiben Satellitzellen und Zellschatten in den regenerierten Ganglien übrig; ferner sind die Ganglien häufig von erheblicher Grösse.

6. Die Nervenfaserbündel sind hypertrophisch und aufgelockert. Ferner können sie eine beträchtliche Unordnung aufweisen. In den einzelnen Fasern sind Verdickungen zu sehen.

7. Die Beobachtungen der Leukozyten im Biopsiematerial zeigen, dass solche bei Patienten, welche die kürzeste Zeit krank gewesen sind, in beträchtlicher Zahl perivaskulär auftreten und dass auch intraganglionär recht bedeutende Mengen derselben zu finden sind. Mit zunehmender Krankheitsdauer geht die Zahl der Leukozyten zurück, und sie sind nur selten anzutreffen, wenn die Krank-

heit länger als einen Monat gewährt hat. Vom Material der Pylorusstenose-Autopsiereihe können keine Schlüsse bezüglich der Leukozyten gezogen werden, da gleichzeitig andere Erkrankungen vorlagen.

8. Es scheint offensichtlich, dass die Zerstörungen im Nervensystem durch pathologische Reize hervorgerufen sind. Es kann dies irgend ein neurotropischer Entzündungsfaktor sein, der in den myenterischen Plexus des Schliessmuskels gelangt. Dies hat erhöhte Nervenaktivität und eine gleichzeitige Phagozytose durch die Leukozyten im entzündeten Gebiet zur Folge. Hinzu kommt der Einfluss der vermehrten Beweglichkeit der Schliessmuskulatur. Das Ergebnis dieser Vorgänge sind Veränderungen der Nervenzellen und Störung der Nerven des Öffnungssystems, wodurch der Öffnungsmuskel ausserstande gesetzt wird, seine Funktion in normaler Weise zu versehen.

9. Die klinischen Symptome sind eine Folge der oben beschriebenen Zerstörung im Nervensystem. Wenn der durch die Entzündung verursachte Reiz zurückgeht, beginnt eine allmähliche Regeneration der Nervenzellen und ein Verschwinden der klinischen Symptome. Die Regeneration erfolgt mit recht geringer Geschwindigkeit, indem noch 3 bis 5 Monate nach Krankheitsbeginn Zerstörung im Nervengewebe wahrnehmbar ist. Die Wiederherstellung wird durch den Umstand verlangsamt, dass nach Aussetzen des Entzündungsreizes der pathologische motorische Reiz noch teilweise vorhält.

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ADYNAMIA EPISODICA HEREDITARIA

BY

INGRID GAMSTORP

Almqvist & Wiksells Boktryckeri AB UPPSALA

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FROM THE PEDIATRIC CLINIC, UNIVERSITY OF LUND
(HEAD: PROFESSOR STURE SIWE, M.D.)

ADYNAMIA EPISODICA HEREDITARIA

BY

INGRID GAMSTORP

LUND 1956

Translated by
Mr. L. James Brown

Printed in Sweden

HÅKAN OHLSSONS BOKTRYCKERI
LUND 1956

To my parents

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I. INTRODUCTION AND PURPOSE OF THE INVESTIGATION

In January, 1952, a girl, aged 14, was admitted to the Department of Pediatrics, Central Hospital, Kristianstad, because of attacks of paralysis of the arms and legs, symptoms which the parents had observed since the girl was about 1 year of age. Sometimes the attacks occurred daily, sometimes at longer intervals and, as a rule, lasted about an hour. The patient's mother and many other members of her family had the same type of symptoms. The clinical picture of the girl thus fitted in best with that of familial periodic paralysis.

During her stay in hospital the girl experienced a few attacks of paralysis during rest after exertion. The attacks occurred during the day-time and lasted about an hour. The following observations of interest were made during the actual attacks: Chvostek's sign was positive, the reflexes were not impaired, the serum potassium was not decreased and the electrocardiogram showed no abnormalities. These observations, the onset at such a low age as one year, and the frequency and transience of the attacks were the first differences noticed between the clinical picture of the girl and that of familial periodic paralysis as described in textbooks of medicine.

Some 10 years earlier Henry Mjönes had seen the same clinical picture in another family. In 1952 Mjönes and I started an investigation of both families which turned out to be branches of the same pedigree.

In the autumn of 1953 a further number of persons with the same symptoms were brought to my notice. These persons also proved to belong to the above mentioned pedigree.

Finally, in co-operation with Danish colleagues, Hauge, Helweg-Larsen & Sagild it was possible to connect a branch that had migrated to Själland to the family, which is referred to below

as the Vånga family after the name of the birthplace of the oldest known member of that family.

A Scanian family with the same type of clinical picture was described by KULNEFF in 1902. Descendants of persons mentioned in that paper were traced. The pedigree was drawn back to the latter part of the seventeenth century without any demonstrable connection with the Vånga family. Below, the family described by KULNEFF is called after the birthplace of the oldest member, the Matteröd family.

From 1954 through 1955 a total of 17 members of the 2 families were examined in the University Hospital of Lund. They were observed between and during attacks, and various provocative, prophylactic and therapeutic methods were tried. The clinical picture proved one and the same in both families. In the course of the investigation the clinical picture could be differentiated clearer and clearer from that of familial periodic paralysis, and in the spring of 1954 an independent disease could be distinguished. This disease was called *adynamia episodica hereditaria*.

Adynamia episodica hereditaria is a genetically determined disease, inherited as a dominant with complete or almost complete penetrance. It is equally common in both sexes. As a rule, it has its onset within the first decade of life. The disease is characterised by frequent – sometimes many a day – attacks of paralysis of the muscles of the trunk and extremities, often accompanied by symptoms from the muscles innervated by the cranial nerves. The attacks last from a few minutes to more than 24 hours, usually up to about an hour. In children the attacks are short; at puberty and during the next decade they frequently become longer and more severe; they then often become milder and less frequent, and after 60 years of age they sometimes disappear. The disease is less troublesome in summer, but, as a rule, more so as soon as the weather becomes cold and damp. The attacks occur at any time of the day, more often during the day-time than at night, particularly during rest after physical exertion. They are also more liable to occur when the patient is hungry. During the attack the serum potassium usually increases without any accompanying decrease in the excretion of potassium in the

urine and with electrocardiographic changes typical of hyperpotassemia. Oral administration of potassium will, as a rule, provoke an attack, calcium administered intravenously will most frequently control an attack, and the administration of glucose before potassium is an effective prophylactic measure.

The disease can be divided into 2 types, and 3 degrees of severity. Below the classification is based entirely on historical data, mainly on the patient's opinion of the disease. Such classification cannot, of course, be more than rough.

Cases with short, well defined attacks without intermittent symptoms can be assigned to the first type. In the second it is not possible to draw such a sharp line of distinction between the attacks and symptom-free intervals: in the course of a couple of hours the paralysis abates sufficiently for the patient to be able to stand and walk, but he still feels weak and he finds it difficult to walk fast or to ascend steps. This weakness can persist for days or weeks. The persistent mild paresis is, as a rule, confined to the muscles of the back and calves and is accompanied by a feeling of stiffness and tension, dull pain and tenderness of the muscles involved.

If the attacks are rare, mild and short, the case is said to be mild. If the attacks are frequent, with widespread paralysis of some hours' duration, the case is said to be severe. The remainder, *i. e.*, the vast majority, are moderate.

As yet no fatal attacks are known. Working capacity is seldom more than slightly impaired.

Part of the present material has been reported on before, namely, the Matteröd family by KULNEFF (1902), the Danish branch of the Vånga family by SAGILD & HELWEG-LARSEN (1955) and part of the Vånga family by GAMSTORP & MJÖNES (1956).

The present paper is concerned with a description of the characteristic features of adynamia episodica hereditaria with special reference to those details in which it differs from familial periodic paralysis, on the basis of information obtained from 138 patients with the disease, 68 of whom were examined personally by the writer.

II. REVIEW OF THE LITERATURE

Periodic paralysis

A clinical picture including acute transient paralysis of the musculature of the extremities and trunk with complete freedom of symptoms between the attacks has been described under several headings.

The first to conceive the syndrome as a clinical entity was WESTPHAL (1885). He called the disease *periodische Lähmung*, and in the German literature the disease is still referred to as such or as *paroxysmale Lähmung* or as *Westphalsche Krankheit*. In the English literature it is known as *periodic paralysis*.

In the German literature WESTPHAL is credited with the first description of the disease. However, DALINGHAUS (1941) referred to some publications from the years 1815–1885, including the first description of the familial occurrence of the disease (SCHACHNOWITSCH 1882).

TALBOTT (1941), on the other hand, ascribed the first description of the disease to MUSGRAVE (1727). Some authors, however, doubt that the case described by MUSGRAVE was really one of periodic paralysis (VIETS 1951).

TALBOTT's and DALINGHAUS' papers contain a detailed survey of all literature on periodic paralysis published until then. Therefore, only few examples from the time before 1941 will be referred to here.

As far back as the beginning of the twentieth century the administration of potassium in periodic paralysis was recommended on purely empirical grounds (MITCHELL, FLEXNER & EDSALL 1902, HOLTZAPPLE 1905).

In 1934 BIEMOND & DANIELS demonstrated decreased serum potassium during an attack of paralysis, a finding confirmed later by AITKEN, ALLOTT, CASTLEDEN & WALKER (1937). This implied the addition of a new characteristic of periodic paralysis and some

authors then referred to the condition as hypopotassemic periodic paralysis. (Berlin 1946, Prader & Zellweger 1952).

Janota & Weber (1928) demonstrated electrocardiographic abnormalities: lower T-waves during the attacks than between them. Voss (1934) described an electrocardiogram, taken during an attack, in which there was, in addition to lower and wider T-waves, prolonged conduction.

In 1941 Talbott collected about 400 cases of periodic paralysis from the literature. His opinion of the disease may be summarised as follows. The triad characteristic of the disease is: periodic paralysis, loss of tendon reflexes and loss of electrical excitability. The familial type of the disease is three times as common among males as among females, and the predilection for the male sex is still stronger in the sporadic type. In the familial cases the mode of inheritance is either recessive or dominant. In some families with periodic paralysis, thyrotoxicosis, epilepsy, migraine, muscle dystrophy or progressive spinal muscle atrophy have been described. As a rule, the disease has its onset within the first two decades of life, most frequently around puberty. Physical exertion and over-eating, especially with exceptional indulgence in carbohydrates, are the commonest precipitating factors. As a rule, the attacks occur during the night. They vary in frequency from one or two a week to anything up to one or two per decade. The mean duration is probably about 6–8 hours. The musculature innervated by the cranial nerves and the respiratory musculature are seldom involved. During the attacks the serum potassium is decreased. The electrocardiograms show low T-waves and wide P–Q, QRS, and Q–T. The administration of potassium exerts a good prophylactic and therapeutic effect. Talbott traced more than 35 cases in the literature in which death had occurred in association with an attack.

According to Dalinghaus (1941), a total of 448 cases (310 males and 138 females) of periodic paralysis had been published up to the end of 1940. Of these, 66 were sporadic (56 males and 10 females). He described the same characteristic triad as Talbott. Dalinghaus found that the trait is usually inherited as a dominant, but with certain exceptions.

DALINGHAUS also found an increased frequency of migraine, epilepsy, muscle dystrophy or endocrine disorders in some families with periodic paralysis. He found the initial attack to occur at any age. The curve he gave for the age at onset shows a maximum peak for 13–15 years and a somewhat lower one for 20–22 years. Both DALINGHAUS and TALBOTT reported 4 families, in which the first attack occurred at a few years of age. (Three of them were, in my opinion, examples of *adynamia episodica hereditaria*. They will be reverted to in a subsequent section.) The most important provocative factors, according to DALINGHAUS, are rest after physical exertion, the ingestion of a carbohydrate-rich meal, exposure to cold, the consumption of alcohol and mental tension. With reference to the low serum potassium found by other investigators during the attacks, DALINGHAUS stated that he found no such decrease in his two personal cases, each of which he examined only once (DALINGHAUS 1941 a). He expressed the view that the best prophylaxis was probably the avoidance of foodstuffs known to be provocative.

During the following years new cases were published, individual symptoms and signs were analysed and the pathogenesis received detailed attention, without, however, adding anything essential to the clinical picture.

The inheritance of periodic paralysis was studied by GAUPP JR. (1940), who described 3 families with a total of 46 cases (24 males and 22 females). He found the mode of inheritance to be that of a dominant trait with complete penetrance. No other diseases of interest occurred among the patients or their relatives. The disease was milder in females than in males. This might account for the higher frequency often found among males in other series. According to GAUPP JR., the disease brings men to the doctor more often than women, and many of the cases thus detected are published. Unless extensive familial investigations are made in every case discovered, males will therefore dominate in the literature.

Two years later GAUPP JR. & KALDEN investigated the inheritance of the disease on the basis of 3 new cases, 2 of which turned out to be familial. Six more cases in 2 and 4 generations, respectively, were found in the 2 families. In these cases the

disease was also inherited as a dominant. The third patient was the point of departure of a familial investigation, which included all the descendants of his maternal and paternal grand-parents, altogether 338, of which 218 were traced. Among these the authors found 37 cases of oligophrenia, epilepsy, migraine, goiter, diabetes mellitus, nephrosclerosis, ulcer, asthma or tuberculosis. But they did not consider the frequency of these diseases or any one of them to be higher in this family than in the general population.

Regular dominant inheritance of periodic paralysis was also described by OLIVER, MCQUARRIE & ZIEGLER (1944) in 16 cases in 3 generations, by JOHNSON (1945) in 9 cases in 5 generations, by ROGER & SCHACHTER (1946) in 7 cases in 4 generations, by CERNY & KATZENSTEIN-SUTRO (1952) in 8 cases in 4 generations, and by BICKERSTAFF (1953) in 6 cases in 3 generations.

Irregular dominant inheritance was described by MYERS (1949) in 25 cases in 6 generations (with 1 omission), by BRISSET (1953) in 7 cases in 5 generations (with 5 omissions), by RACHELSON (1954) in 5 cases in 2 generations (with 1 omission), and by SAGILD & HELWEG-LARSEN (1955) in 50 cases. The last-mentioned authors expressed the view that the penetrance is less complete among females than among males.

The occurrence of chronic changes in the musculature of the patients or their closest relatives was described by PENARD (1942), MYERS (1949) and BICKERSTAFF (1953); of epilepsy, by MEYER (1952); of migraine, by MYERS (1949) and BICKERSTAFF (1953); of Reynaud's disease, by CERNY & KATZENSTEIN-SUTRO (1952); and of kyphoscoliosis, by MÜLICH (1949).

In typical cases of periodic paralysis the onset occurs in the second decade of life. This has been confirmed by several investigators such as HOLMES (1941), GAUPP JR. & KALDEN (1942), MCGEORGE (1942), PENARD (1942), CURSCHMANN (1946), JANTZ (1947), MÜLICH (1949), SOYSA (1949), CERNY & KATZENSTEIN-SUTRO (1952), BICKERSTAFF (1953), RACHELSON (1954), BECCUAU, VELLUZ, DELGA & COIRAULT (1955), HOSOTTE, FERRAND, PHILIPPON, WIDLOCHER & SOULAYROL (1955). SAGILD & HELWEG-LARSEN (1955) found the onset to have occurred between the ages of 14 and 20 in 35 of 50 patients. Exceptions are, however, on record. Cases in

which the onset occurred between the ages of 7 and 10 were described by GAUPP JR. (1940), GAUPP JR. & KALDEN (1942), SILVERSTEIN (1944), JANTZ (1947), MEYER (1952), and after 20 years of age by SHINOSAKI (1926), GAUPP JR. (1940), GAUPP JR. & KALDEN (1942), MCGEORGE (1942) and HAMMES (1951). Families, in whom the onset of the disease occurred between the ages of 4 and 31 were described by OLIVER et al. (1944); and between 8 and 31 years, by MYERS (1949).

The frequency of the attacks varies widely. Even in one and the same family it may vary from 1 to 200 attacks a year (OLIVER et al. 1944, MYERS 1949). Attacks at intervals of weeks to months were found to be the rule by SAGILD & HELWEG-LARSEN (1955).

In his series of 28 patients MYERS (1949) found the duration of the attack to vary between 1 and 96 hours (average 12 hours). OLIVER et al. (1944) reported a similar variation. A duration of 6 to 48 hours was given by SAGILD & HELWEG-LARSEN (1955).

The discovery in 1934 by BIEMOND & DANIELS of low serum potassium during attacks prompted a number of investigations of the potassium metabolism in periodic paralysis. AITKEN et al. (1937) confirmed this observation and showed that if potassium was administered during an attack, the serum potassium increased with decreasing paralysis. They produced attacks by lowering serum potassium by the administration of glucose. Even in apparently healthy individuals the administration of glucose caused a decrease in the serum potassium, but not so marked as in patients and it was not accompanied by paralysis. ALLOTT & MCARDLE (1938) claimed on the basis of metabolic studies that potassium is retained in the body during attacks.

FERREBE, ATCHLEY & LOEB (1938) showed during a metabolic investigation lasting for six months: that the serum potassium decreases during attacks; that potassium chloride administered by mouth is an effective prophylactic and therapeutic measure; that the excretion of potassium in the urine decreases during attacks, independently of the amount of urine excreted; that the attack is not related to the blood sugar level or the serum phosphorus; and that the patient has permanent creatinuria, which increases by anything up to 50 per cent during attacks.

Pudenz, McIntosh & McEachern (1938) showed that potassium administered intra-arterially during attacks into an arm shut off from the general circulation had no effect on the weakness of the arm. Potassium injected intravenously into the other arm, however, increased the strength of both arms, even though one was shut off from the circulation. On the basis of these observations the authors concluded that the potassium exerted its effect via the central nervous system.

Gammon, Austin, Blithe & Reid (1939) noted that the serum potassium regularly decreased during attacks, but they found no correlation between the severity of the paralysis and the magnitude of this decrease. They found hydration to provoke attacks in their patient.

Stewart, Smith & Milhorat (1940), who examined their patient during attacks, found low serum potassium and electrocardiographic abnormalities: low, wide T-waves, wide P–Q, QRS, and Q–T. On administration of potassium by mouth the electrocardiogram soon became normal but the paralysis did not disappear until 9 hours later, by when the serum potassium spontaneously had returned to normal.

Electrocardiographic changes of the same type during an attack of periodic paralysis were described by Perelson & Cosby (1949).

Jantz (1947) traced 23 different theories suggested to explain the pathogenesis of periodic paralysis. His own material consisted of 9 patients examined during 68 attacks. In that material he regularly found a decrease in the serum potassium, the decrease varying with the severity of the attack without increase in the excretion of potassium in the urine or faeces, and an abnormally large amount of potassium in the muscles during one attack studied in one patient. The serum calcium, sodium, and magnesium showed no changes. The serum phosphorus and the blood and urinary creatine decreased during attacks. The administration of glucose or DOCA provoked attacks. Oral administration of potassium chloride in aqueous solution regularly controlled the paralysis within 1–2 hours, but it had no prophylactic effect.

Danowski, Elkinton, Burrows & Winkler (1948) investigated the potassium and sodium metabolism in periodic paralysis. They

found that during development of the paralysis the potassium passed from the extracellular fluid to the intracellular with an abrupt fall in the extracellular potassium, which in turn elicited the paralysis. During recovery the increase in the concentration of potassium in the extracellular fluid was preceded by a further increase in the concentration of the potassium in the intracellular fluid.

Gass, Cherkasky & Savitsky (1948) found the excretion of potassium in the urine to be strikingly low when the attack reached its maximum during which the serum potassium fell.

Ziegler (1949), who studied a patient during 2 mild attacks, found the serum potassium to be within normal limits (16 mg/100 ml), and during 2 more severe attacks in the same patient lower (10 mg/100 ml). The attacks were elicited by the administration of carbohydrates and occurred during the early hours of the morning, *i. e.*, when the patient had been resting for 8–12 hours after the ingestion of carbohydrates. Attempts to provoke an attack by hydration failed. The administration of potassium by mouth controlled the paralysis within 2 hours, but intravenous injection of potassium produced no demonstrable effect.

Lundbæk (1949) found that 100 g of glucose administered after over-eating with indulgence in carbohydrates produced a typical attack, but not when the patient had avoided dietary carbohydrates before the experiment.

A Norwegian family with a typical clinical picture and good response to potassium therapy was described by Valsö (1951).

In two articles Ziegler & McQuarrie (1952) and McQuarrie & Ziegler (1952) published the results of a long-term metabolic investigation of the effect of dietary factors and supply of potassium on the paralysis. In repeated experiments they gave 150 g of glucose, which two hours and a half later produced an attack with simultaneous decrease in serum potassium. The paralysis reached a maximum at the same time as the serum potassium was lowest. Five grams of potassium chloride controlled the paresis within 40 minutes. No increase in potassium losses was demonstrable (Ziegler & McQuarrie 1952). With a high-protein, moderate-fat and moderate-carbohydrate diet such a small amount

of potassium that the ratio carbohydrate : potassium was at most 75 : 1 was sufficient to prevent attacks. Any increase in the amount of fat at the expense of proteins required an increase in the supply of potassium to a carbohydrate : potassium ratio of 10 : 1, if the patient was to be kept free of symptoms. Hydration with or without pitressin provoked no attacks. The administration of 61 grams of NaCl and 8 liters of fluid in the course of a day provoked paralysis with a decrease in the serum potassium (McQuarrie & Ziegler 1952).

Cerny & Katzenstein-Sutro (1952) found an increased excretion of the follicle stimulating hormone during attacks and a tendency to a decreased excretion of this hormone and of the 17-ketosteroids between attacks. They also found that the serum potassium was decreased during paralysis and that potassium had a good therapeutic effect. They stressed the close resemblance between the attacks of periodic paralysis and Selye's "general adaptation syndrome". They interpreted the prodromal stage of the attack as adrenocortical hypofunction and the actual attack as an excessive response of the anterior pituitary. They believed the fundamental cause of the disease to be lability in the area of the hypophysis-hypothalamus.

In a patient with periodic paralysis McArdle (1954) could provoke a severe attack by intramuscular injection of 25 mg ACTH when the diet contained an ordinary amount of potassium. When the potassium content of the diet was increased, the injection of ACTH produced only slight muscle weakness. During the first few hours after the injection the excretion of potassium in the urine increased to decrease so much later during the attack that potassium was retained. The amount of exchangeable potassium, as determined with K^{42} during a free interval while the patient was on a high-potassium diet, was found to be normal.

Beccuau, Velluz, Delga & Coirault (1955) found the intravenous administration of 20 mg of adenosine triphosphate during an attack of periodic paralysis with low serum potassium to produce a prompt effect. The administration of 3 mg of adenosine triphosphate 3–4 times daily by mouth had a certain prophylactic effect. The essential disorders, according to these

authors, were due to disturbed synthesis of adenosine triphosphate and phosphocreatine.

EICHLER, JANTZ & JUNG (1940) who carried out electromyographic studies during the development and abatement of spontaneous attacks and attacks produced with insulin-glucose found the essential change to consist of elongation of the summation potential induced by electrical stimulation of the nerve. This change occurred before, and persisted after, the clinically demonstrable paralysis and decrease in serum potassium and was regarded by them as being due to a decreased velocity of propagation of the impulse over the muscle. On the basis of these investigations the authors found the disease to be due to a disturbed mineral metabolism in the muscles.

GAMMON & HARVEY (1941) found that during an attack the electromyographic potentials were of small amplitude and practically monophasic. They assumed that the impulse did not propagate at ordinary velocity over the muscle fibres and concluded that the disease was localised to the muscles.

PETERSÉN & WIDÉN (1955) studied the electromyographic changes during an attack of periodic paralysis with hypopotassemia in a sporadic case in a woman, aged 60. The pattern was of normal appearance: despite paresis, they found a large number of potentials, and no spontaneous fibrillation potentials. The duration of the potentials was decreased. An increase was noted in the number of polyphasic potentials. During treatment with potassium chloride the clinical condition of the patient improved and the duration of the potentials increased. The serum potassium was hardly influenced by the treatment and was still low after clinical recovery.

Normal electroencephalograms between and during attacks were reported by WATSON (1946), HAMMES (1951) and SAUNDERS (1954). The patients of the 2 last-mentioned authors had decreased serum potassium during the attack, while in WATSON's case no such decrease was demonstrable.

TALBOTT (1941) found the clinical picture to be the same in familial and sporadic cases. JANTZ (1947), who observed no difference in the reaction pattern of 7 cases of familial type and 2 of sporadic type, shared this opinion.

Decreased serum potassium and/or electrocardiographic changes of the same type as in the familial cases and/or the beneficial effect of potassium therapy have been described in cases of sporadic type (STOLL & NISNEWITZ 1941, L'ORANGE 1945, BERLIN 1946, RADERMECKER & DE HAENE 1946, GROSSMAN 1949, HYLAND 1949, HOFMANN 1952, PRADER & ZELLWEGER 1952, WEISSMAN 1952, BLOMBERG & LINDQVIST 1954, GOODMAN 1954, BECCUAU et al. 1955, COUSTON 1955, PAINTER 1955).

Descriptions of typical cases, in which the electrolyte balance and the electrocardiogram were, however, not studied, were presented by JOHNSSON (1931), for example, in whose patient Babinski's sign was demonstrable during an attack, by KIRK & MÖLLER (1933), HOFMAN-BANG (1941), FASANARO (1947), SKALWEIT (1948), KAMMAN (1949), LENZ (1950), and HOZAY (1951).

Cases in which the attacks were probably precipitated by infection have been presented by WESTPHAL (1885), STRAUSS (1932), KVINT (1933) cit. CERNY & KATZENSTEIN-SUTRO (1952), L'ORANGE (1945).

Disturbances in the hypophysis-hypothalamus region have been reported by POULSEN (1938), GROSSMAN (1949), PRADER & ZELLWEGER (1952), while COUSTON (1955) described periodic paralysis in a mentally defective boy, injured at birth.

BREDEMANN (1952) published a case in which sporadic periodic paralysis and dystrophia myotonica occurred at the same time.

In sporadic cases the most common predisposing factor reported in the literature is thyrotoxicosis. This has been pointed out especially in Japan, where sporadic periodic paralysis, especially its combination with exophthalmic goiter appears to be more common than elsewhere (SHINOSAKI 1926, TSUJI 1939). Simultaneous thyrotoxicosis and sporadic periodic paralysis have, however, also been described elsewhere such as by DUNLAP & KEPLER (1931), MORA (1932), MORRISON & LEVY (1932), SEED (1947), KEPNER (1951), COGSWELL & BEATON (1952), HOFMANN (1952), WEISSMAN (1952), ROBERTSON (1954), LINDER (1955).

Even in patients with an inherited predisposition for periodic paralysis thyrotoxicosis can have a provocative effect (HILDEBRAND & KEPLER 1941).

Cases with a clinical picture resembling adynamia episodica hereditaria

Of the many reports on periodic paralysis in the literature, some with a somewhat deviating clinical picture regarded by the writer as presumably being examples of *adynamia episodica hereditaria* were selected. The characteristics of the disease were described in brief in the introduction.

a) Buzzard (1901) described an English family, in which the mother and her 2 sons had frequent attacks – sometimes lasting for a couple of hours – of paralysis since childhood. The attacks always occurred during rest after exertion, and they were refractory to potassium therapy.

b) This material includes some of the cases described by Kulneff (1902) under the heading of myatonia periodica.

c) Schoenthal (1934) described a family in the U. S. A., in which 18 members (8 males and 10 females) suffered from attacks of paralysis. Heredity was simple dominant, no generation was skipped. Characteristic of this family was that the attacks began in early childhood, in several members already at the age of 1 year. In addition, the attacks occurred at any time of the day, with a frequency of up to several attacks per day, each attack lasting at most an hour except in 1 patient, in whom it occasionally lasted for 24–48 hours. The disease showed a tendency to abate after the age of 30.

Wolf (1943) re-examined 7 of Schoenthal's cases and found that none of them responded to potassium therapy but all to thyroid medication. The serum potassium was determined during 4 attacks and was found to be within normal limits in 1 and to be increased in the other 3 (28, 29 and 35 mg/100 ml).

Stevens (1954) continued the investigations of the same family as Schoenthal and Wolf. It had in the meantime grown to include 23 cases (10 males and 13 females) of attacks of paralysis in 5 generations. The new cases showed the same clinical picture, early onset, short frequent attacks, refractory to potassium. Peculiar to this family was also the occurrence of other neuromuscular

disturbances: progressive muscular atrophy with electromyographic signs of neurogenic injury, areflexia of the lower extremities also between attacks, sensibility disturbances, pes cavus, hypertrophy of the calf muscles, myotonia and in 1 myasthenia gravis. One or more of these symptoms were noted in 8 members with, and 3 without, attacks of paralysis. Microscopic examination of a muscle biopsy specimen from 1 patient showed a picture similar to that seen in dystrophia myotonica. The patient had incipient cortical cataractous changes, but no atrophy of the testes. None of the other patients examined showed evidence of cataract.

d) WYLLIE & WATKINS (1948) described a family in Wales with 19 cases of periodic paralysis in 5 generations. In 2 the onset was given as 3 years and as 8 months, respectively. Potassium therapy tried in 1 case was followed by further progression of the disease.

e) LEWIS (1950) described a 38 year old Chief Petty Officer in the British navy, who was able to carry on with his work, despite the fact that he had transient attacks of paralysis since early childhood. The attacks occurred during rest after exertion, usually commencing after about an hour's rest, and lasted for 1–2 hours. The administration of potassium to this patient and to an affected relative produced no improvement. Altogether 10 cases (5 men and 5 women) of the disease had occurred in the family in the last 4 generations without any of them being skipped.

f) TYLER, STEPHENS, GUNN & PERKOFF (1951) of Salt Lake City reported on a family with 33 members with attacks of paralysis in 7 generations. The type of inheritance was that of a dominant trait with complete penetrance, males and females being affected equally often. Attacks of paralysis usually appeared at 1 year of age, but had sometimes been observed in children only a few months old. The duration varied between a few minutes and a day. Rest after physical exertion provoked attacks, but the ingestion of ample amounts of carbohydrates did not, although it produced a decrease in the serum potassium. During the attacks the serum potassium level was normal (3.5 to 5.4 mEq/l when the attack was at its maximum), and no electrocardiographic abnormalities occurred. The condition was refractory to potassium. Microscopic examination of a biopsy specimen from the muscles

of the calf showed vacuolisation of the fibres. TYLER et al. suggested the possible existence of two or more types of familial periodic paralysis.

g) ECKER & CARSON (1953) described attacks of paralysis of the muscles of the extremities, trunk and face with loss of muscle reflexes in a girl, aged 9 years. The disease was first observed when the child was 3 months old. The attacks lasted from 5 minutes to one hour and a half and varied in frequency from once a day to once every sixth week. The attacks occurred during rest after exertion and could not be provoked by the administration of glucose or glucose in combination with insulin. The serum potassium, which was determined during one attack only, was found to be somewhat higher than during a free interval (5.3 and 4.9 mEq/l, respectively). Of 5 electroencephalograms traced, all except one were of abnormal appearance. An electromyogram, the only one taken, showed no abnormalities. Treatment with potassium produced no effect. The patient's mother, brother and sister had the same trouble, and all 3 showed electroencephalographic abnormalities. The maternal grand-father, who was living in Esthonia, had probably had the same disease.

h) SAGILD & HELWEG-LARSEN (1955) published parts of the present material (15 patients) under the name of *periodic adynamia* and stressed the following differences from periodic paralysis namely, regular dominant inheritance, frequent attacks of short duration, no hypopotassemia during attacks, and poor response to the administration of potassium. One of their patients was described for the first time by NEEL in 1928.

i) Three other cases merit mention as possible examples of adynamia episodica hereditaria. GILLESPIE (1937) in U. S. A. described a boy, aged 5, who had suffered from attacks of paralysis since the age of 18 months. The attacks occurred during rest after exertion. They occurred several times a day and lasted for 10–30 minutes. No other case was known in the family.

BENEDEK & VON ANGYAL (1942) of Budapest described a male patient who had had attacks of paralysis since 6 years of age. He usually had attacks twice a week, each attack lasting 6–8 hours. The serum potassium was not decreased during attacks. He was

treated for some time with 2.5 g of potassium chloride daily, during which, however, the attacks appeared twice as often as before, and on one occasion he was found to have hyperpotassemia (34 mg/100 ml) during an attack. No other case was known in the family.

BENEDEK & VON ANGYAL (1943) described another male patient with attacks of paralysis who became worse on administration of potassium. During attacks the serum potassium lay between 16.8 and 28.4 mg/100 ml. The clinical picture gradually resembled that of dystrophia musculorum progressiva. A sister had had the same trouble, but no other cases were known in the family.

Hyperpotassemic paralysis

Here mention will be made of only a few examples of neurologic symptoms and signs in hyperpotassemia.

In 1915 SMILLIE described a woman with a renal disease, who developed paresthesia and muscle weakness after the administration of potassium.

Hyperpotassemia with paresis can occur if the excretion of urine is decreased, especially if the patient has received extra amounts of potassium (FINCH & MARCHAND 1943, FINCH, SAWYER & FLYNN 1946, NICHOLSON & SPAETH 1949, KOLFF 1950, MERILL, LEVINE, SOMERVILLE & SMITH 1950, MCNAUGHTON & BURCHELL 1951, CREEVY & REISER 1952, BULL, CARTER & LOWE 1953, RICHARDSON & SIBLEY 1953).

In untreated Addison's disease paresis also sometimes occurs (BULL et al. 1953, MARKS & FEIT 1953, RICHARDSON & SIBLEY 1953, FELTS 1954).

Among other causes of hyperpotassemia mention might be made of; crush syndrome and hemolytic reactions (BYWATERS 1944, HOLLEY & CARLSON 1955), hyperkinetic activity (HIRSCHFELDER & HAURY 1948, ENDE, BRAZDA & ZISKIND 1952), decreased extracellular fluid (BERNREITER 1954).

The administration of 5–15 grams of potassium by mouth to apparently healthy individuals will often produce paresthesia

(ARDEN 1934, KEITH, OSTERBERG & BURCHELL 1942, DODGE, GRANT & SEAVEY 1953, BEDFORD 1954). Muscle weakness, on the other hand, is rare and has been described in exceptional cases after the administration of 7–15 grams of potassium by ARDEN (1934), DODGE et al. (1953), BEDFORD (1954).

III. MATERIAL

Collection

The Vånga family. – As mentioned in the introduction, I learned of the existence of some of the branches of the family through the intermediary of Swedish and Danish colleagues (IX: 67 through Mjönes and VIII: 13 through Hauge, Helweg-Larsen & Sagild). The other two branches (IX: 95 and IX: 100) were discovered by the writer.

The parish registers contain no notes about the disease, and all data collected about the earlier generations are based on hearsay evidence passed down from one generation to another. After a family branch had been drawn back as far as possible by such personal inquiry among the surviving members, the parish books were resorted to, and the branch was traced back until it could be linked to some known earlier branch or branches. In this way the pedigree of the Vånga family was drawn back to Jöns Månsson (II: 1), born on December 18, 1699, in the parish of Vånga, and his wife, Elna Nilsdotter (II: 2), born in November, 1706. The names of the parents of this couple are also known. Information about the birth-place of Elna Nilsdotter was unavailable.

It was not possible to trace the family back any further. Then, likewise with the aid of the parish register, all later generations were studied in an attempt to trace as many descendants as possible from Jöns Månsson and Elna Nilsdotter. The purpose of this procedure was to find and interview at least one of the members of each branch. A person's knowledge of his family usually embraces only his grandparents and their offspring. Therefore, when the link to the large pedigree dated back further than two generations, as counted from the oldest traceable surviving member, I tried to find a representative of every individual family of that branch. After representatives had been traced in

the parish registers, I visited them personally and inquired about their state of health and that of their relatives. In those branches in which attacks of paralysis had occurred, the persons interviewed immediately recognised the description of the symptoms and readily gave the names of those members of the family that had or had had the disease. In this case I continued the investigation mainly on the basis of information obtained from the personal interviews, after which I got into touch with all of the families where one or more of the members were known to be affected. In the other branches in which the disease was unknown, the members were surprised by my inquiry. These branches were then deleted as from the earliest member known by the interlocutor.

In this way I got to know 122 affected persons (including the 4 probands) and 221 unaffected parents, sibs and children of the affected members, each person being accounted for only once. About 1,000 persons were registered in the pedigree.

It was not possible to trace all of the descendants of Jöns Månsson and Elna Nilsdotter. For one of the following reasons some 50 members of the family could not be traced and had not been heard of for such a long time that no information was available about their state of health:

They had left the country.

They had on some unknown grounds been cancelled from the parish registers.

They had not been registered in the parish to which they had moved according to the registers in their former place of domicile.

They had been transferred from the parish register to the "Book of untraceable citizens".

The parish books had been destroyed.

The Matteröd family. — As mentioned, the pedigree of the Matteröd family is based on KULNEFF's (1902) paper. The persons referred to in that paper and their descendants were traced with the aid of the parish books. In addition, a further branch could be linked to the family tree, a patient cared for at the Department of Medicine, Central Hospital, Kristianstad, under the diagnosis of familial periodic paralysis (see page 27).

In KULNEFF's paper Johannes T, Matteröd, born on January 10

1826, was described as the earliest known ancestor with the disease. All of his known descendants living in Sweden were traced.

In this way I became acquainted with 16 affected persons (including the 10 given by Kulneff) and 31 unaffected parents, sibs and children of the affected members, each person being accounted for only once.

Johannes T's maternal and paternal ancestors were traced back as far as possible, *i. e.*, to the latter part of the seventeenth century (one branch, however, only to 1715). During this period no connection was found between the Vånga and the Matteröd families.

Johannes T's sibs died childless and, according to Kulneff, without ever having been troubled by attacks of paralysis. Kulneff also stated that Johannes T's parents were not affected. In order to secure as high a degree of certainty as possible on this point I traced the descendants of the sibs of Johannes T's parents. About 150 persons were registered in this part of the investigation. At least one representative of every individual family was traced and interviewed. None of them had symptoms of adynamia episodica hereditaria and none of them had ever heard of such symptoms. The results of this study, then, did not argue against Kulneff's statement that Johannes T was the first in his family to have attacks of paralysis.

Hospital registration. – The annual reports issued during the 1900–1950 period by all departments of medicine and pediatrics in Skåne and Blekinge were searched for cases diagnosed as periodic paralysis. Three cases were traced. Of these, 2 were already known to the writer from the familial investigation. By inquiry into the familial history of the third, who had been treated at the Department of Medicine, Central Hospital, Kristianstad, an earlier unknown branch could be linked to the Matteröd family. (This patient's number in the pedigree is IV: 13.)

Source of error. – The diagnosis of adynamia episodica hereditaria was based entirely on what the interlocutors reported about themselves and their relatives (except in the 21 cases examined also during attacks, see pages 54 and 57). This might have involved

a certain source of error, some of the persons questioned possibly having given misleading information, though not necessarily on purpose. Those interviewed were, however, well informed about their relatives and co-operated willingly. Therefore the errors can be regarded as small.

Table 1. *Material*

State of health: a = affected, u = unaffected, x = unknown.

	Patients	Sibs[1]		Parents[2]			Children		
		u	x	a	u	x	a	u	x
Total	138	144	24	65	68	11	124	167	8
Family									
Vånga	122	129	24	57	58	11	109	151	8
Matteröd	16	15	—	8	10	—	15	16	—
Sex									
Male	73	77	7	31	36	5	69	91	3
Female	65	67	17	34	32	6	55	76	5
Manner of disappearance from observation									
Living Dec 31, 1954	99	100	—	35	35	—	96	132	—
Dead	34	39	12	26	26	11	25	31	2
Untraceable	5	5	12	4	7	—	3	4	6
Age at disappearance from observation									
0—4	2	16	6	—	—	—	2	26	1
5—9	8	15	—	—	—	—	8	25	—
10—19	26	20	4	—	—	—	26	26	2
20—29	21	17	7	8	4	—	20	18	4
30—39	23	12	1	9	14	—	22	15	—
40—49	19	18	—	11	15	—	15	16	—
50—59	10	16	3	10	12	5	8	14	1
60—69	15	16	—	12	6	—	13	16	—
70—79	8	7	1	9	5	2	5	6	—
80—89	5	4	1	5	6	3	4	4	—
90—99	1	2	—	1	—	—	1	—	—
Age unknown	—	1	1	—	6	1	—	1	—

[1] Including half-sibs (9 persons: 4 unaffected, 5 state of health unknown), whose common father or mother belongs to the Vånga family.

[2] Every mating is accounted for separately. Three persons are represented twice (included in 2 matings), 1 person 3 times (included in 3 matings).

Not accounted for in the Vånga family: the parents of a group of half-sibs, state of health of the mother unknown, nothing known about the fathers.

Composition

The total material consisted of 138 affected persons and 252 unaffected parents, sibs and children. All data given hereinafter refer to the situation at the end of 1954.

A survey of all the individual families included in the investigation is apparent from Table A (page 108). The 2 pedigree charts are given on pages 112 and 113.

Table 1 gives a survey of the data available about the patients, their unaffected sibs, their parents and children, according to family, state of health, sex, age at, and manner of, disappearance from observation.

The number of affected children of affected fathers was 62 (33 males and 29 females); of affected mothers, 62 (36 males and 26 females), while as to 14 affected (4 males and 10 females), none of the parents were known to be affected.

The Vånga family included one first cousin marriage. However, the common ancestors of these couples did not belong to the family. This figure is based on data given by the patients and not

Table 2. *Patients examined at University Hospital of Lund*

Patient's No.	Pedigree No. V = Vånga M = Matteröd	Sex	Age in years Dec 31, 1954	Type (see page 9)	Severity A = mild B = moderate C = severe
1	V IX:95	F	16	1	B
2	V IX:88	M	11	2	B
3	V IX:91	F	11	2	B
4	M VI:4	F	10	1	B
5	M VI:5	M	4	1	B
6	M V:3	F	28	2	B
7	V IX:97	M	20	2	B
8	V X:70	M	10	1	B
9	V X:71	M	5	1	A
10	M IV:13	M	60	2	C
11	V X:1	M	16	1	A
12	M IV:9	M	60	1	B
13	V IX:47	M	20	2	C
14	M V:4	M	26	2	B
15	V IX:66	F	20	1	B
16	V IX:100	F	37	2	B
17	V IX:62	M	31	2	A

on an investigation of the parish registers for any consanguineous marriages.

Of the affected members, 17 (Table 2) were examined at the University Hospital, Lund.

Since the clinical picture was the same in both families, they will be taken together in the description given below.

Controls

The control material consisted of apparently physically healthy children admitted to hospital for observation, of probationers and of one apparently healthy member of the Vånga family (IX: 49). The age and sex distributions of the controls are given in Table 3.

Table 3. *Controls*

Control	Sex	Age in years at examination	Control	Sex	Age in years at examination
A	M	9	K	F	22
B	M	10	L	F	21
C	M	11	M	F	22
D	M	11	N	F	21
E	M	11	O	F	23
F	M	10	P	F	26
G	M	12	Q	F	21
H	M	10	R	F	22
I	F	21	S	M	24

Control S was an apparently healthy member of the Vånga family (IX:49 in the pedigree).

IV. METHODS

Subjects not examined in hospital

Patients

Personal examination. — Fifty-one of the patients were interviewed at their homes. Careful inquiry was made into the history of their disease and that of their affected children. They were also questioned about symptoms of any neurologic or endocrine disorders they or their relatives had or had had as well as about any relationship between their parents. The neurologic investigation included: examination for atrophy or hypertrophy, assessment of the gross functional strength of the extremities, examination of the tendon reflexes and pupillary and plantar reflexes. All patients were examined during a free interval.

Four of these patients received 4 g potassium chloride orally in the fasting state, which regularly precipitated an attack, (Vånga VIII: 4, X: 2, X: 15 and X: 17). In this way it was possible to compare the state of the patient during a free interval with that during an attack.

Including the 17 patients examined in hospital altogether 68 patients were examined personally.

Information by questionnaires. — Six patients living far away from the hospital were questioned by letter.

Information from close relatives. — This group consisted of those who had died before the beginning of the investigation or who were untraceable and included 9 patients who were away from home when called upon. The data about 36 of the patients were based on information from close relatives.

Information from other investigators. — Information about 9 patients was obtained from KULNEFF's paper. The result of the investigation of 13 patients was placed at my disposal by Mjönes and 6 patients by Hauge, Helweg-Larsen & Sagild. Thus, data about 28 cases are based on information from these investigators.

Apparently healthy relatives

In connection with the field investigation 9 healthy relatives (Vånga X: 3, X: 4, X: 5, X: 16, X: 69 and a daughter of VII: 7, Matteröd IV: 10, IV: 12, V: 2) received potassium chloride. Two of them (Vånga X: 4, X: 5, 9 and 10 years old, respectively) received 3 g and the remaining 7 received 4 g by mouth before breakfast and were examined in the same way as the patients. In addition, one unaffected member of the Vånga family (IX: 49) was included as a control and tested with potassium chloride.

Patients examined in hospital

Of the 17 patients, 16 were admitted to the University Hospital of Lund, while one (No. 14) was examined 2 days at the Outpatient Department.

The history was inquired into in the same way as in the field investigation. Clinical investigation included neurologic examination, and special ophthalmologic examination at the Eye Department, Lund. The electric excitability was tested indirectly with galvanic and faradic current on the fibular nerve.

The patients were examined between and during attacks, and various provocative, prophylactic and therapeutic methods were tried. In the first 3 patients admitted, attacks were precipitated only by rest after physical exertion; in most of the others, also by oral administration of potassium or by both methods in combination.

Laboratory studies. — The erythrocyte sedimentation rate was determined, the blood picture was studied and electrocardiograms were taken routinely. The urine was examined for protein, reducing substance, urobilinogen, hemoglobin and formed components. In many cases the basic metabolic rate, non-protein nitrogen, Wassermann reaction were studied, and in 3 the reaction to the ACTH-test. In 3 patients the cerebrospinal fluid was also examined.

The potassium and sodium in the serum, urine and cerebrospinal fluid were determined in a Beckman Flame Photometer, model B. Determinations were made on 0.1 ml of the respective

fluids by the method of CHANEY (1952). The serum determinations were made on venous blood when possible, *i. e.*, in most examinations of adults and older children, otherwise on capillary blood. In the fasting state the potassium content of venous blood and arterial blood differs only immediately after short, intense physical exertion (FARBER, PELLEGRINO, CONAN, EARLE 1951). Therefore, in the present investigation no distinction was made between the results obtained with venous blood and capillary blood.

Capillary blood was taken from the tip of a finger. Venous blood was collected from a cubital vein with stasis without the patient opening and clenching the hand, and was allowed to flow directly into a centrifuge tube. All samples were allowed to stand at room temperature until the blood had coagulated and cooled down, *i. e.*, at most 40 minutes before they were centrifuged for 10 minutes, immediately after which the serum was separated off. When serum and erythrocytes are separated off so soon after collection of the sample, the escape of the potassium from the erythrocytes to serum is negligible (GOODMAN, VINCENT & ROSEN 1954).

In order to assess the reliability of the single observation with the technique and approaches used, the following experiments were carried out: blood was collected in a series of 10 tubes from one volunteer and in the same way from another. The 20 tubes were afterwards mixed and numbered by a disinterested person. The samples were centrifuged after at most 40 minutes, and the serum potassium and serum sodium were determined. Twelve series of 10 samples each were studied, 6 of venous blood and 6 of capillary blood.

The serum calcium was determined by the method of NIELSEN (1952). By means of this technique and the same reagent as used for the determination of the serum calcium, the amount of calcium plus magnesium was determined by titration without previous precipitation of the calcium, and the difference taken as the amount of magnesium (GRETTE 1953). Since 2 ml of serum was used for each examination, only venous blood was employed. In the evaluation of the reliability of a single observation corresponding examinations were made as for potassium and sodium. Because

of the large amounts of blood necessary only 5 samples were used for each series. Seven series of 5 samples each (plus one of only 2 samples) were carried out.

The serum phosphorus was determined by the method of MAGNUSSON & SYLVAN (1929). The reliability of the single observation was determined in the same way as for potassium and sodium.

On the basis of these examinations the standard error of the single observation was found to be (bracketed figures denote percentage of mean value)*:

Serum potassium	in venous blood	0.10 mEq/l	(2.5 per cent)
	in capillary blood	0.12 mEq/l	(2.7 per cent)
Serum sodium	in venous blood	1.0 mEq/l	(0.7 per cent)
	in capillary blood	1.3 mEq/l	(0.9 per cent)
Serum calcium		0.07 mEq/l	(1.4 per cent)
Serum magnesium		0.09 mEq/l	(6.8 per cent)
Serum phosphorus		0.28 mg/100 ml	(7.2 per cent)

Serum chloride was determined by the method of BRUN (1949).

The serum bicarbonate was determined by van Slyke's manometric method (1932) on 0.1 ml of serum.

The blood sugar was determined by the method of HAGEDORN, HALSTRÖM & NORMAN JENSEN (1935).

For the assessment of the hematocrit blood was taken in 5 cm long tubes rinsed with heparin, and centrifuged for 20 minutes at 4000 r. p. m. The values given are means of double determinations.

The number of eosinophils was determined according to the method of RUD (1947).

The 24-hour excretion of creatine and creatinine in the urine was determined by the method of FOLIN (1904) and FOLIN & WU (1919).

For determining the potassium excreted, urine was collected during 2–5 hours during the day-time and 7–12 hours at night. On the basis of data about the amount of urine, time, and potassium content, the excretion of potassium in mEq/hour was calculated. When possible, the excretion of potassium was determined

* The statistical calculations were performed by Tage Larsson, M. D.

both during an attack and during a free interval. The excretion of potassium showed a wide diurnal variation. In the next chapter the values given for the excretion of potassium during a free interval refer to samples collected during the morning of a day without attacks, *i. e.*, the same time as when attacks were otherwise precipitated.

The urinary 17-ketosteroids were determined at the Hormone Laboratory, Malmö General Hospital.

Other examinations. – The intelligence quotient was judged according to Healy II and in 1 case according to the CVB scale (*Civila Värnpliktsbyrån*).

Testing with d-tubocurarine and succinylcholine chloride was done in co-operation with the anesthesiologist, Dr. Bertil Löfström. At least 24 hours were allowed to elapse between the two tests, which were carried out during a free interval with the patient in the fasting state. The patient received d-tubocurarine intravenously in intermittent doses. Succinylcholine chloride in the form of 0.1 per cent solution in saline was given by adjustable intravenous drip.

V. RESULTS

The two families were traced back to two parishes about 50 km apart from one another in the north of Skåne.

The birth places of 135 of the 138 patients are plotted in Fig. 1. Three of them were born further north: 1 in Stockholm, 1 in Västergötland and 1 in Dalarna.

At the end of 1954 thirty-four of the patients were dead and 5 were untraceable. Of these 5, 4 had migrated to the U. S. A. Attempts to trace them there were unsuccessful.

The places of domicile of the remaining 99 patients at the end of 1954 are given in Figs. 2 and 3.

Of the 138 patients, 73 were males and 65 females. Thus, generally speaking, the disease is equally common among males and females.

Etiology

All of the cases were hereditary. No other etiologic factor was demonstrable.

It is apparent from the pedigree charts that the disease occurred in both families in 5 generations without skipping. In addition, according to hearsay, in one of the branches of the Vånga family the disease had presumably occurred in members of 2 earlier generations.

Such uninterrupted inheritance through 5, possibly 7, generations can only be interpreted as dominant.

In all of the cases in which information was available about the state of health of the patients' parents, the patients were offspring of the mating affected × unaffected. Only as to 1 individual in the entire material, Vånga VIII: 16, was one generation possibly skipped. Her mother was affected and among her 9 children 4 were also affected. Judging by information obtained

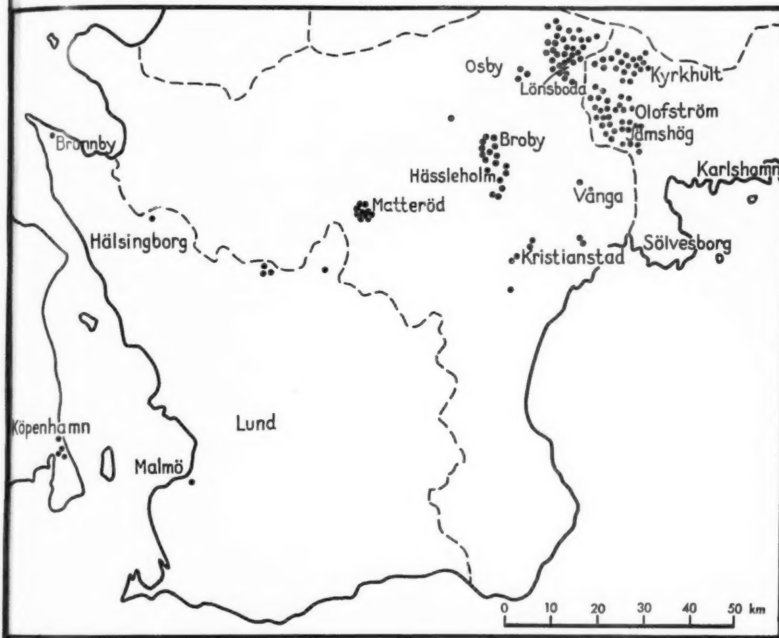

Fig. 1. Places of birth of 135 of the patients.

from her brothers and sisters and from her children she was probably unaffected. Since she had died many years before, it was not possible to decide whether she really was healthy or whether she might have had the disease in a mild form, possibly only during part of her life. This member was classified under the heading "state of health unknown".

In this connection it might be mentioned that another woman (Vånga VIII: 67) was unaffected until she was 31 years of age, by when her son had shown symptoms of the disease for a few years.

In autosomal dominant inheritance, half of the children of matings affected × unaffected inherit the disease and males and females are affected equally often. The number of affected and unaffected children in 83 families with adynamia episodica here-

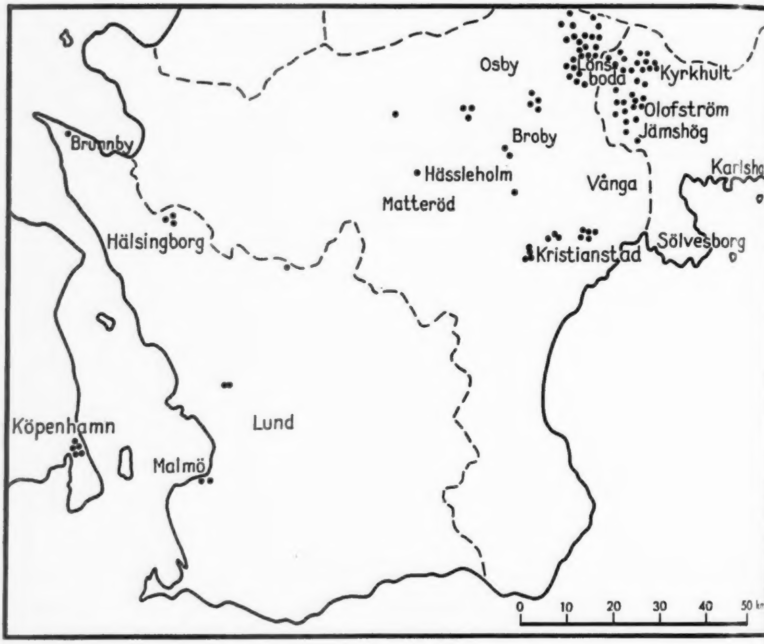

Fig. 2. Places of domicile of those patients who were alive at the end of 1954 (see also Fig. 3).

ditaria, and where information was obtained about all of the members are given in Table 4. The table shows not only the entire number of children, but also those aged 5 years or more and those aged 10 or more, since it must be taken into account that some of the children included in the table as unaffected, have not yet reached, or have died before, the age at which the disease manifests itself.

The age at onset of the disease is accounted for in a later section (see table 7, page 48). It is apparent from this table that in about half of the patients the disease manifested itself before they had reached the age of 5 years and in more than 90 per cent below 10 years. Therefore this age was taken as a limit,

Fig. 3. Places of domicile of those patients who were alive at the end of 1954 (outside the area covered by Fig. 2).

Table 4. *Families, state of health of all members known. Parent mating: affected × unaffected.*[1]

	Number of families	Total number of children		Number of children ≧ 5 years of age		Number of children ≧ 10 years of age	
		affected	unaffected	affected	unaffected	affected	unaffected
Total	83	121	164	119	138	111	113
Family							
Vånga	73	106	148	106	128	98	103
Matteröd	10	15	16	13	10	13	10
Sex							
Male		67	89	66	75	61	59
Female		54	75	53	63	50	54

only members at least 10 years of age being included in the following calculations. In the Matteröd family there were thus 13 affected and 10 unaffected children from affected × unaffected matings, the corresponding figures for the Vånga family being 98 and 103. In neither family did the values observed differ more than slightly from the expected 1 : 1 ratio. When both families were taken together the ratio was almost ideal, namely 111 : 113. The ratio affected : unaffected was 61 : 59 for males and 50 : 54 for females.

Thus, from affected × unaffected matings there were, practically speaking, just as many affected as unaffected children 10 years of age or more, and males and females were affected equally often.

Further evidence of the inheritance being dominant is provided by the fact that the disease occurred among half-sibs, whose common parent was affected (or belonged to the pedigree, though state of health was unknown). That one affected individual belonging to 2 different matings had affected children in both, occurred once in the Matteröd family (V: 3) and twice in the Vånga family (VI: 13, IX: 13). That one affected individual

[1] Two matings affected × unaffected belonging to the Vånga family not included. In one there were 6 children: 2 affected, 3 unaffected, 1 state of health unknown. In the other family there were 8 children: 1 affected, 7 state of health unknown. One individual (state of health unknown) was below 10 years of age, all others above.

belonging to 3 different matings had affected children in all. occurred once in the Vånga family (VIII: 45). Finally, the Vånga family included one woman (VI: 1) of unknown state of health, mother of a group of presumably half-sibs, of whom 2 were affected.

Only with respect to one individual did the disease possibly skip a generation. Penetrance is thus complete or almost complete.

In dominant, totally sex-linked inheritance the gene responsible for the disease is situated in the non-homologous part of the X chromosome. When the gene is rare, all daughters but no sons of affected fathers will have the disease, which will be twice as common among females as among males. In Y-linked or holandric inheritance all sons of an affected father will have the trait, but no daughters; the disease is not observed in females. As far as adynamia episodica hereditaria is concerned, these modes of inheritance are excluded by the equal sex-distribution and by the occurrence of the disease among both sons and daughters of affected fathers.

In dominant, partially sex-linked inheritance the gene is situated in the homologous part of the sex chromosome, in some individuals in the X chromosome, in others in the Y chromosome. With such a mode of inheritance as with autosomal the sex distribution in a given material is equal, but analysis of individual families will reveal a difference.

Since children of both sexes inherit an X chromosome from the mother, no conclusions can be made about the sex distribution among the children of affected mothers. The father transmits his X chromosome to all his daughters and his Y chromosome to all his sons. In view of this inheritance of the chromosomes, the affected children of an affected father are usually of the same sex, either only boys or only girls and the unaffected of the other sex.

Since males inherit the X chromosome from the mother and the Y chromosome from the father, partially sex-linked inheritance is also characterised by affected children of affected fathers being usually of the same sex as the affected paternal grand-parent.

Relevant findings made in adynamia episodica hereditaria are given in Tables 5 and 6. For reasons given earlier, only children

Table 5. *Families, state of health of all members known, at least 2 children over 10 years of age, at least one affected.*

	Mating: affected father × unaffected mother				Mating: unaffected father × affected mother
	Families in which all affected children were males	Families in which all affected children were females	Families with affected children of both sexes	Total	Total
Number of families	7	6	11	24	22
Sons (≧10 years of age)					
affected	10		20	30	26
unaffected	4	3	8	15	34
Daughters (≧10 years of age)					
affected		9	13	22	20
unaffected	6	7	8	21	22

10 years or above were included. Since families with only 1 child are non-informative with regard to sex distribution they are not included in Table 5. Twenty-four families fulfilled the requirements for inclusion in the table, *i. e.*, father affected, at least 2 children above 10, at least one of them affected. In 13 of these families all of the affected children were of the same sex; in 11, of both sexes. In 7 families in which all of the affected children were males, there were also 4 unaffected sons and in 6 in which all of the children affected were females there were also 7 unaffected daughters. It is apparent from Table 6 that of 45 affected children of affected fathers, 23 were of the same sex as the affected paternal grandparent and 22 of different sex.

Table 6. *Children over 10 years of age. Mating: affected father × unaffected mother.*

Children	Affected	Unaffected
Of like sex with affected paternal grand-parent	23	21
Of unlike sex with affected paternal grand-parent	22	14

Therefore, dominant, partially sex-linked inheritance can be excluded.

The inheritance of adynamia episodica hereditaria is thus due to a single dominant autosomal gene with complete or almost complete penetrance.

Relation to other diseases

Only in a few cases were other neurologic or endocrine disorders observed in the two families. The Vånga family included one patient (IX: 22) operated on because of toxic goiter, one (VIII: 17) with atoxic goiter and one (VIII: 22) with migraine. Member (IX: 22) who was interviewed by letter reported nothing about any effect of the operation on the attacks of paralysis. The hospital records of this member contain no notes about these attacks. Among the unaffected members one (VIII: 39) had had epilepsy.

The Matteröd family included 2 members (IV: 9 and IV: 13) both hospital cases, with permanent slight loss of strength of the extremital muscles, in the latter accompanied by muscle atrophy.

These data are based mainly on information given by the members about themselves and their relatives and therefore cannot be regarded as complete. It is nevertheless evident that other neurologic and endocrine disorders were not more common in the 2 families than in the general population.

Pathology

Those patients who had been examined *post mortem* had died from some intercurrent disease, and no special search had been made for changes possibly peculiar to adynamia episodica hereditaria.

Muscle tissue was taken from 3 patients (examined in hospital: Nos. 10, 13 and 16) and examined histologically.*

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* The histologic examinations were carried out by Professor Gunnar Wohlfart.

Figs. 4–9. Case 10. Biopsy specimen from the anterior tibial muscle.

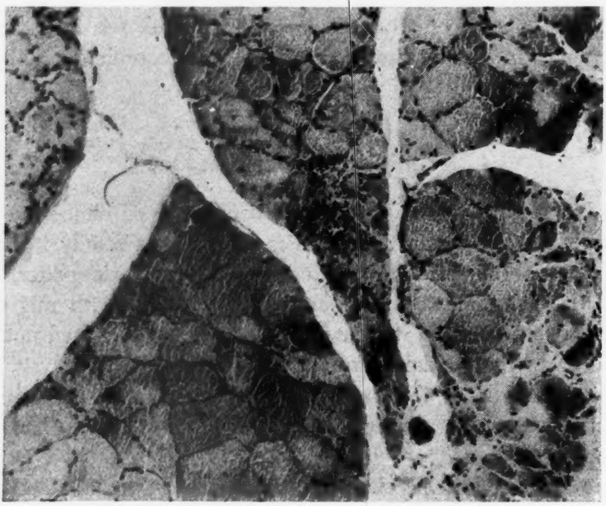


Fig. 4. General view. Hematoxylin-eosin × 100.

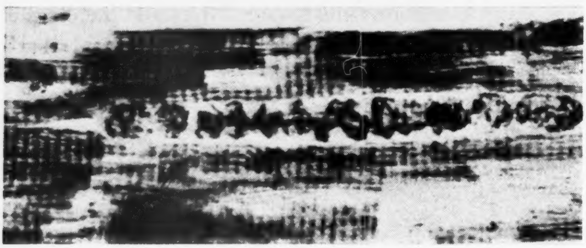


Fig. 5. Davenport silver impregnation × 900.
The figure demonstrates a row of nuclei in the centre of a muscle fiber.

The specimens from patients 13 and 16, aged 20 and 37, respectively, showed no signs of a pathologic condition. Neither of these patients had atrophy, and between attacks there was no

Fig. 6. Hematoxylin-eosin × 450.

Cross-section through muscle fibres with striated annulets (Ringbinden).

muscle weakness or reflex disorders. On the other hand, the third patient, aged 60, had slight atrophy of the muscles of the lower leg and thigh, and even between attacks the extremities were weak and the Achilles tendon reflexes absent. A muscle biopsy specimen from the anterior tibial muscle showed the following features (Figs. 4–9).

"The piece of muscle examined showed considerable pathologic changes. All of the muscle fibres were pathologically changed. A few of the muscle fibres were hypertrophic, others were of normal size, and many had undergone a varying degree of atrophy. Several of the extremely atrophic fibres had fallen into pieces, which were filled with clusters of nuclei. In cross section normal and severely atrophic muscle fibres were irregularly intermingled. In all of the muscle fibres hypolemmal nuclei had migrated into

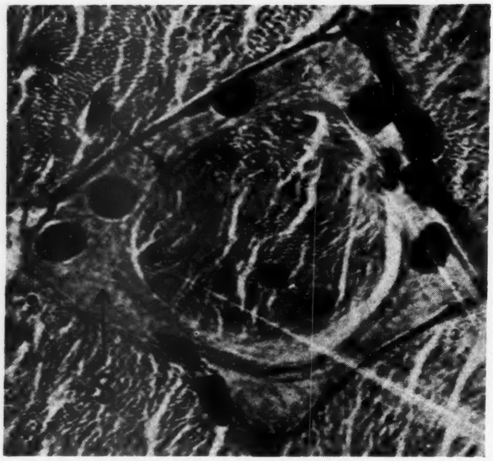


Fig. 7. Hematoxylin-eosin × 900.

Cross-section through a muscle fibre with a peripheral layer of sarcoplasm. "A" indicates a thin, striated annulet.
"S" indicates the peripheral sarcoplasm layer.

the central parts of the fibres. These nuclei were often seen in long rows in longitudinal sections.

Many of the muscle fibres showed a peripheral disintegration of the myofibrils. In these fibres were large peripheral layers of sarcoplasm and numerous circular, striated myofibrils ('striated annulet', 'Ringbinde'). A few fibres were in state of vacuolar degeneration.

There was a considerable increase in interstitial connective tissue with incipient fatty infiltration. No interstitial cell increase was seen. One muscle spindle found in the specimen showed no pathologic changes. The tissue examined contained no intramuscular nerve trunk.

The intramuscular vessels were of ordinary appearance.

Diagnosis: Changes of the type seen in dystrophia myotonica (Prof. G. Wohlfart)."

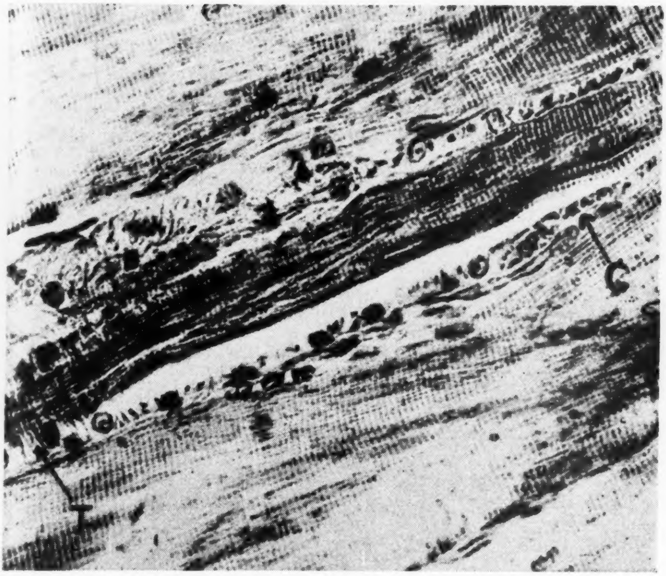


Fig. 8. Davenport silver impregnation $\times$ 450.

Longitudinal section through a muscle fibre with a peripheral layer of circular, striated myofibrils ("striated annulet", Ringbinde).
"C" indicates cross-cut myofibrils in the striated annulet.
"T" indicates tangentially cut myofibrils in the striated annulet.

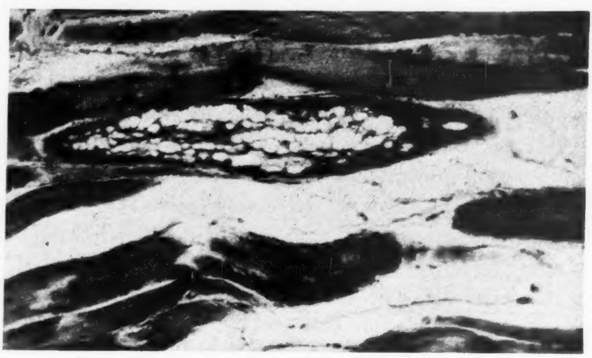


Fig. 9. Davenport silver impregnation $\times$ 100.
Vacuolar degeneration.

It should, however, be pointed out that the course of the disease in this patient differed from that usually seen (see page 96) and that this was the only one of the 68 patients examined personally that showed muscular atrophy with certainty. Therefore, the question whether the histologic changes observed were related to adynamia episodica hereditaria must be left open.

Anamnesis

The clinical data about the entire material are summarised in Table B (page 114).

Age at onset. – Only in exceptional cases could the patients or their parents remember exactly when the first attack occurred. Besides, the disease is not readily recognised in young infants. The age at the time of the initial onset is therefore accounted for in 5-year periods (Table 7). Patients who reported that they had had the disease as long as they could remember or when they began to walk, were allotted to the 0–4 year age class. In 30 cases, in which the persons had died or were untraceable, no information at all was available about the age at onset.

The mean age at onset of the disease in the remaining 108 patients was 6.0 ± 1.8 years (for males 5.4 ± 1.8 and for females 6.7 ± 1.7). The lowest age at onset was 8 months (Matteröd VI: 5) and the highest 31 years (Vånga VIII: 67).

Table 7. *Age at onset*[1]

Age at onset in years	Number of patients	Males	Females
0—4	48	30	18
5—9	51	24	27
10—14	5	2	3
15—19	3	2	1
30—34	1	—	1
Total	108	58	50

[1] No information available about the age at onset in 30 cases.

Table 8. *Type and severity*[1]

Severity	Number of patients	Type 1		Type 2		Not typed	
		M	F	M	F	M	F
Mild	19	7	8	1	2	1	—
Moderate	65	13	19	16	11	4	2
Severe	6	—	—	4	1	1	—
Total	90	20	27	21	14	6	2

Type and severity (for definition see page 9). – The distribution of the patients according to type and severity of the condition is apparent from Table 8. The data in the table refer to the state of the patients during the field investigation in 1952–1954. Neither in type nor in severity were the symptoms constant throughout life.

Symptoms between attacks. – Patients of type 1 were symptom-free between the attacks. Patients of type 2 had the same symptoms as patients of type 1, but in addition sometimes prolonged trouble. A few hours after the onset of an attack the symptoms abated so much that the patients could stand and walk with exertion, but they often felt weak for days and weeks. The weakness was mainly localised to the back and calves and was accompanied by dull pain, tenderness and stiffness of the musculature. The prolonged symptoms occurred mainly during the winter months.

One male patient of type 2 (Matteröd IV: 13), who is now 60 years old, has had permanent slight paresis of the extremities for the last 10 years.

Another male patient, aged 60 (Matteröd IV: 9), has also slight permanent weakness of the arms. In him, however, the attacks are well defined and do not last more than one hour, and he is not troubled by any muscle pain or tenderness. Therefore, although he is not completely symptom-free between attacks, in the present investigation he was included among the patients of type 1.

The attack. – All of the patients reported that the attacks were precipitated by rest after physical exertion. Rest appears to be a

[1] In 48 cases type and severity could not be assessed.

necessary condition for the elicitation, because a limb is never paralysed as long as it is exercised. The duration of rest before the onset of the attack varied between a few minutes and hours, although it was usually about an hour. Although rest after a severe physical exertion more regularly elicited an attack and then, as a rule, of greater severity, preceding slight physical exertion was also often enough.

The initial symptom of the attack was a feeling of heaviness of the limbs. If the patients continued to rest, the paralysis gradually reached a maximum, which occurred some minutes to an hour after this initial symptom. The paralysis persisted at a maximum for a varying, but usually short, period and then abated at roughly the same rate as it developed. The frequency and duration of the attacks varied from a few attacks a day to one a year and from a few minutes to a few days. Ninety-four patients of different ages or their parents were able to say, at least roughly, how frequent and how long their attacks generally were. (See Tables 9 and 10.)

The data in the tables refer to information obtained from the patients during the field investigation (1952—1954) on the

Table 9. *Frequency of attacks*[1]

Age in years Dec 31, 1954	Number of patients	Several daily		At least once daily		At least once weekly		At least once monthly		At least once yearly	
		M	F	M	F	M	F	M	F	M	F
0—4	1	1	—	1	—	1	—	1	—	1	—
5—9	7	1	—	2	2	3	3	4	3	4	3
10—19	22	2	2	5	5	7	9	10	12	10	12
20—29	13	2	1	3	2	7	5	8	5	8	5
30—39	21	2	1	4	4	6	7	7	9	10	11
40—49	14	—	—	3	1	4	5	6	6	7	7
50—59	5	2	—	2	—	2	1	3	2	3	2
60—69	9	—	—	—	—	5	1	5	3	5	4
70—79	2	—	—	1	—	1	1	1	1	1	1
Total	94	10	4	21	14	36	32	45	41	49	45

[1] The data refer to time of field investigation (1952—1954), or concerning patients who were free from attacks at that time, to the period they last had attacks.

No information available about the frequency of attacks in 44 cases.

Table 10. *Duration of attacks in hours*[1]

Age in years Dec 31, 1954	Number of patients	$\leq \frac{1}{2}$		≤ 1		≤ 2		≤ 4		≤ 24		≤ 72	
		M	F	M	F	M	F	M	F	M	F	M	F
0—4	1	1	—	1	—	1	—	1	—	1	—	1	—
5—9	7	3	1	4	3	4	3	4	3	4	3	4	3
10—19	22	4	7	9	10	10	11	10	12	10	12	10	12
20—29	13	—	2	2	4	5	5	7	5	7	5	8	5
30—39	21	3	4	8	9	8	11	9	11	10	11	10	11
40—49	14	1	2	4	7	7	7	7	7	7	7	7	7
50—59	5	1	—	1	1	3	2	3	2	3	2	3	2
60—69	9	—	2	2	4	2	4	2	4	4	4	5	4
70—79	2	—	—	—	1	—	1	—	1	1	1	1	1
Total	94	13	18	31	39	40	44	43	45	47	45	49	45

approximate frequency and duration of their attacks during the last few years, or, as to those who were at that time free from attacks, the frequency and duration during the last period they had had attacks.

It is apparent from the tables that about three fourths of the patients had at least one attack a week and about the same number reported that the attacks lasted for at most one hour.

The spread and severity of the paralysis varied from one patient to another and from one attack to another. The entire attack could consist of a transient feeling of heaviness in the legs or slight weakness of the hand; it could also be more severe and could even be so severe that the patient was helpless and unable to lift the head from the pillow or to lift a hand or a foot. As a rule, it was the legs that were first and most severely paralysed, but all muscles of the extremities and trunk could be affected, although the respiratory musculature was seldom involved, and then only slightly. Seven patients of 91 reported that they had found it difficult to cough and to take a deep breath during at least one severe attack, but stated that they never had respiratory distress. Micturition and defecation were not disturbed. Symptoms from

[1] The data refer to time of field investigation (1952—1954), or concerning patients who were free from attacks at that time, to the period they last had attacks.

No information available about the duration of attacks in 44 cases.

the musculature innervated by the cranial nerves were reported by about half of the patients as having occurred on at least one occasion. They complained of difficulties in swallowing, in articulation and in moving the eyes. Double vision was reported by one, ptosis by none.

If the patients exercised the muscles at the first symptom of an attack or as soon as they could during or after a fully developed attack, it accelerated the abatement of the symptoms. They could walk off their paralysis. All the patients reported that moderate exercise was the best procedure to prevent or to shorten the duration of an attack.

In patients of type 2 maximum possible exercise during the attacks accelerated the abatement of the most severe paralysis, but the attacks were then more liable to be followed by prolonged symptoms in the form of mild paresis, dull pain and muscle tenderness.

The intake of food also influenced the course of the attacks. Fifty-six patients reported that they were liable to have attacks when they were hungry and that the intake of food, especially bread, had a favourable prophylactic and therapeutic effect. They could, so to say, ward off or eat off the paralysis. The other 28 patients interviewed stated that the attacks were not influenced by food.

In the first stage of the attack the patients were often troubled by mild paresthesia of the hands and feet and face. These symptoms disappeared when the paresis reached its maximum. There were no other sensibility disturbances. The attack had no effect on the level of consciousness.

The attacks, particularly when severe, were sometimes accompanied by clammy sweating and general discomfort. The patients were, however, troubled most by their inability to move the limbs. Passive movement of the limbs gave a certain amount of relief.

Difference between sexes. — The disease showed a tendency to be more severe among males than among females. It appeared earlier in life, and the attacks were somewhat more frequent and more prolonged (Tables 7, 9 and 10). This is also apparent from Table 8, in that 5 male cases were classified as severe, as against

only 1 female, though there were almost as many male cases as female judged as slight. Type 2, which was characterised by prolonged, ill-defined attacks and discomfort also between attacks, is more troublesome to the patient than type 1. Type 1 was more common among females; type 2, among males. The number of observations in each of these respects was not large enough to permit any valid conclusions. However, the available data conveyed the impression that the disease was somewhat more severe among the males.

Variation with age. – In childhood the attacks were short. In puberty they became longer and more severe, but did not change appreciably in frequency. The condition was, as a rule, most troublesome between the ages of 15 and 30. Afterwards the attacks often decreased in frequency and severity without any definite change in duration. About half of the patients above 30 reported an improvement with age. This improvement was to a certain extent due to the fact that the patients gradually learned how best to avoid attacks, although true improvement could not be excluded. Of 11 patients over 60 years of age, 3 reported that they had had no attack during the last few years.

According to information obtained during the field investigation, patients below 10 years of age had never had attacks lasting more than 1 hour. Furthermore, patients below 30 years had an attack at least once a month (Tables 9 and 10).

Seasonal variation. – Of 94 patients, 64 reported that the condition was most troublesome in winter, especially during the winter months after Christmas. They also stated that cold and damp weather had an unfavourable influence on the condition, no matter whether in summer or in winter.

Diurnal variation. – All the patients had had attacks during the day-time, 72 had also had one or more attacks at night, while 27 had never had nocturnal attacks. Nocturnal attacks were liable to occur in the event of physical exertion shortly before retirement for the night. Then the patients awakened after a few hours' sleep and were unable to move. The severity of the attack, spread and duration varied within the same limits as attacks during the day-time.

Variations with menstruation and pregnancy. — All of the patients reported that the disease was most troublesome during their teens, although none of the females reported any particular progression at the time of menarche. Only in 3 was the condition reported as exacerbating in connection with menstruation. Twelve reported a distinct deterioration during pregnancy, while 15 stated that they had noticed no change. One patient had had attacks during 2 of her 3 pregnancies, but otherwise never (Vånga VIII: 67). Only one reported improvement during pregnancy (Vånga VIII: 4).

Findings at examination

Domiciliary examinations

Patients. — Examinations between the attacks revealed nothing of interest except atoxic goiter in one patient (Vånga VIII: 17).

Attacks were provoked in 4 patients aged 13–62 by oral administration of 4 g potassium chloride. The attack started 30–45 minutes after the administration and lasted 15–30 minutes in the three youngest (13–24 years), and 90 minutes in the oldest. In one of them the attack consisted of slight symmetric weakness of the extremities, but she could stand and walk without support. Two had more severe symmetric paresis of the extremities and could not stand or walk without support. In all these three reflexes were normal. The fourth had mild left hemiparesis. The reflexes were less brisk on the left side than on the right. Chvostek's sign was negative in all. The musculature was not tender and was normal to palpation. Neither the musculature innervated by the cranial nerves nor the respiratory musculature was involved. One of the patients had facial paresthesia, but otherwise no sensibility disturbances were noted in this group, consciousness was never impaired and they all felt well.

Apparently healthy relatives. — In none of the 10 relatives who stated that they were healthy and who received potassium chloride was the administration followed by any symptoms. The gross functional power of the extremities was normal, and no disturbances of the reflexes were demonstrable.

Patients examined in hospital

Seventeen of the patients were examined between attacks and during attacks provoked in different ways. The age, sex and number in the pedigree, and the type and severity of the disease are given in Table 2.

EXAMINATION BETWEEN ATTACKS

Clinical examination revealed no physical or neurologic findings of interest in 14. One patient (No. 15) was in the fifth month of pregnancy at the time of the examination. In the oldest 2 patients, aged 60, the gross functional power of the extremities was found to be decreased, even during intervals between attacks. One (No. 12) of them had slight weakness of the arms and decreased hand strength but no signs of atrophy or reflex disturbances. The other (No. 10) had slight paretic and atrophic muscles of the calves and thighs, decreased hand strength and weakness of the extensor muscles of the arm. The changes were symmetric. The Achilles tendon reflex was absent bilaterally. No other neurologic signs were observed.

One of the 17 patients (No. 17) was being cared for at the Department of Psychiatry because of insufficientia depressiva.

Laboratory studies. — The erythrocyte sedimentation rate and blood picture examined in 16 cases revealed nothing of interest. Of the blood electrolytes, the serum potassium was determined in all, the serum sodium in 16, the serum calcium in 6, the serum magnesium in 2, the serum chloride in 8 and the serum phosphorus in 7. In addition, the blood sugar was determined in all, the hematocrit in 15, blood N. P. N. in 4, the serum bicarbonate in 8. All of the values found lay within normal limits. Wassermann reaction in the blood was carried out in 7 cases and was negative throughout.

Heller's test and the urinary sediment were examined in all, Almén's test was carried out in 16 cases, examination for urobilinogen in 6 and benzidine in 5: the results were normal throughout.

Electrocardiographic studies of all of the patients revealed

nothing of interest. The glucose tolerance test was done in 15 cases and all the curves were normal. The basic metabolic rate was determined in 13: in one (No. 16) it was –14 per cent, in the remainder it was normal for age. The ACTH-test was done in 3 cases with normal results with subsequent fall in the circulating eosinophils: 112–25, 656–6, 212–75. The excretion of 17-ketosteroids in the urine was determined in 12 patients, in all of whom it was found to lie within normal limits for sex and age.

Examination of the cerebrospinal fluid obtained by lumbar puncture in 2 patients revealed nothing of interest. Wassermann's test in the cerebrospinal fluid in 1 gave a negative result.

Other examinations. – Ophthalmologic examination of 16 patients revealed no cloudiness of the lens or malformations.

Electroencephalograms were taken in 4 cases. The tracings were normal in all.

In 8 the intelligence quotient was determined and the values were found to lie between 80 and well above 100.

Five patients (Nos. 8, 10, 11, 13 and 16) were tested with d-tubocurarine. They first received 0.01 mg per kg body-weight. None of them reacted to this dose.

Within about 4 minutes a second dose was given, bringing the total dose up to 0.1 mg per kg body-weight. This second dose was followed by ptosis, double vision, feeling of heaviness and slight weakness of the extremities in all. Patients No. 10 and 16 could lift the head from the pillow without difficulty, the others only with exertion.

Three (Nos. 10, 13 and 16) of the patients received a further dose after 4–6 minutes. This dose was such as to bring the total dose received within 10 minutes up to 0.20–0.25 mg per kg body-weight. After this dose none of them could lift the head or the extremities and impaired thoracic respiration was seen in all.

The reaction of the test subjects to d-tubocurarine lay within normal limits (Killian & Weese 1954).

Four patients (Nos. 10, 11, 13 and 16) were tested with succinylcholine chloride.

These patients first received 20–30 micrograms per kg body-weight per minute. After five minutes' administration at this rate

double vision and a feeling of mild heaviness of the extremities developed in all. They could lift the head from the pillow. Respiration was not affected.

The dose was increased to 50–75 micrograms per kg bodyweight per minute. After 2–5 minutes' administration at this rate none of them could lift the head from the pillow and respiration was impaired in all. Within 5 minutes of the conclusion of the test all symptoms disappeared.

The reaction of the patients to succinylcholine chloride lay within normal limits (Espinosa & Artusio 1954).

In 2 patients (Nos. 13 and 16) an attack of adynamia episodica hereditaria developed within half an hour after the test. In one (No. 16) the attack was mild, and passed off spontaneously in about 20 minutes. In the other patient the attack was severe and prolonged. It abated after intravenous administration of calcium, but did not disappear completely until after about 24 hours. The attack was, however, not longer or more severe than those to which the patient was accustomed. Since rest and hunger are the two commonest provocative causes, the administration of succinylcholine chloride might not have been responsible for these two attacks, although the possibility cannot be excluded.

EXAMINATION DURING ATTACKS AND PROVOCATION

The clinical findings and the results of blood studies and electrocardiography in 61 tests on 17 patients are summarised in Table 11. Fifty-eight attacks were precipitated. In 3 tests prophylactic measures had such a good effect that no attack was precipitated. Three more attacks in which the patients were examined clinically, but in which no laboratory studies were made, are not included in the table.

Terminology

Precipitation

I. Rest after exertion. After having walked or played out-of-doors or run up and down steps for 20–120 minutes the patient lay still on an examination table until the attack came and abated. All cases precipitated in this manner were classed as I.

II. Oral administration of 2–5 g potassium chloride. No physical exertion before or during the attack. All cases precipitated in this way were classed as II.

III. In one patient (No. 12) attacks were precipitated by a combination of I and II, so that during rest after physical exertion he received 4–5 g potassium chloride by mouth. The two attacks precipitated in this way were classed as III.

All attacks referred to as I, II or III were precipitated in the morning before breakfast.

IV. All attacks observed at different times of the day when the patient was resting, but not in the fasting state, were classed as IV. The reason why these attacks were not classed as I was that the patients were not in the fasting state, that the attack was not preceded by any particular physical exertion and that the testing and recordings during the various phases of the attack could not be done with the same accuracy as in the tests classed under heading I.

When a patient was observed during more than one attack or after provocative measures, the tests are distinguished by small letters given after the patients' number.

Severity

Three degrees were distinguished according to the severity when paralysis was at a maximum. In the mildest attacks (grade 1) the patient could lift the extremities against light resistance, he could sit up, stand and walk. In the moderate attacks (grade 2) the patient could turn on the examination table, lift the head from the pillow, move the hands and feet but not lift the extended legs from the table, not stand or walk. In the severe attacks (grade 3) the patient was completely helpless, he could not move on the examination table, he could not lift the head from the pillow, or move the hand or the foot on the table. Grade 0 indicates that no attack occurred.

Time

Table 11 gives the duration of 3 different phases. The first figure indicates the interval from the end of physical exertion or the time of administration of potassium until the beginning of the attack; the second figure, until the attack reached its maximum; and the third, the duration of the actual attack. Unless otherwise stated, all times are given in minutes. The occurrence of symptoms in the form of a feeling of heaviness of the extremities, *i. e.*, symptoms usually appearing before any measurable decrease in the functional strength, were said to indicate the onset of the attack. When the paralysis was most severe the attack was said to have reached a maximum. The end

of the attack is to be understood as the time by when the patient had completely recovered. The best defined moments in this course are the time of the end of physical exertion or administration of potassium and the time the attack reached a maximum: therefore, this interval was included in the table. On the other hand, the duration of the actual attack is of such great interest that it was included despite the fact that it is difficult to time the onset and the end of the attack with exactitude. For completion, the first interval in the course, the interval between the end of physical exertion or administration of potassium and the initial symptoms of the attack is also given.

In those cases in which the attack lasted more than one day, the paresis abated so much within 1–3 hours that the patient could stand and walk, but he did not recover completely until after 1–3 days. In reality, the transition between the various phases of the attack is not so sharp as might be suggested by the figures given in the table. Maximum paralysis usually lasted some minutes, but sometimes as long as half an hour without any appreciable fluctuation. In such cases paralysis was said to have reached a maximum in the middle of this period.

General Discomfort

The plus-sign indicates the occurrence of a certain general discomfort, when the attack was at a maximum, *i. e.*, one or more of the following symptoms: cold sweat, nausea, general unrest, irritability, depression; minus, the absence of any of these symptoms.

Reflex Disturbances

The plus-sign indicates weakening or absence of one or more of the tendon reflexes; minus, unchanged reflexes.

Involvement of the Musculature Innervated by the Cranial Nerves

The plus-sign indicates the occurrence of symptoms of musculature innervated by the cranial nerves (facial paresis, swallowing difficulty); minus, the absence of any such symptoms.

Chvostek's Sign

The plus-sign indicates positive Chvostek; minus, negative.

Blood Studies

The serum potassium was regularly determined before precipitation of attacks I, II and III. The serum potassium was determined again at

the end of physical exertion and then again every half hour after the end of exertion or administration of potassium until paralysis appeared and disappeared, or — when the attack lasted more than 24 hours — only 2 to 4 hours after the attack had reached its maximum. The entire series of determinations made for 8 attacks are given on pages 75 to 83. The other series showed the same regular pattern; therefore only values referable to 3 determinations are included in the table.

The first value, referred to below as the initial value, was determined before the physical exertion or the administration of potassium on the morning of the day the test was carried out. The second value, referred to below as the culmination value, was determined during or — since culmination could occur between the collection of 2 samples — at most 15 minutes before or after the culmination. The third potassium value in the table indicates the culmination value minus the initial value, and the fourth designates the initial value minus the post-attack value (post-attack value is to be understood as the determination made at the end of the attack or, if the attack was prolonged, when the symptoms were abating).

As to the other blood determinations, only 2 values are given: the initial value and the culmination value, both made at the same time as the corresponding potassium determinations. However, the last eosinophilic count was made during or up to 3 hours after culmination of the attack (see page 64).

Electrocardiographic Studies

The plus-sign indicates the occurrence of electrocardiographic changes (see page 64); minus, the absence of any such changes.

Remarks

Here the type and the duration of exertion and/or the dose of potassium chloride are given.

Clinical Findings

A total of 61 attacks were observed in 17 patients. The clinical picture did not differ with the manner of precipitation. The main symptom was flaccid paresis, commencing with a feeling of heaviness of the extremities, increasing to a maximum and afterwards spontaneously abating. As a rule, the paralysis began in the muscles of the legs and spread within a short time to the arms and trunk, sometimes in the muscles of the arms, and occasionally in

the back and hip muscles. The respiratory muscles were not involved.

No abnormal reflexes appeared, the tendon reflexes were never increased and clonus was not noted; the pupillary and plantar reflexes were normal.

The musculature was not tender and was normal to palpation. Increased mechanical irritability of the type seen in myotonia was not noted. Response to galvanic and faradic stimulation of the fibular or median nerve was normal.

A common symptom during the initial phase of the attack was paresthesia of the face, the hands and feet. These symptoms disappeared when the attack reached its maximum. No other sensibility disturbances were noted.

Consciousness was always preserved.

A relatively slow regression and long duration as compared with the earlier attacks described by the patients were observed. This can be explained by the fact that contrary to habit, they lay still without food throughout the attack instead of trying to work or eat it off.

Twenty *attacks I* were precipitated and allowed to abate spontaneously. In addition, in 11, various therapeutic measures were tried. Thus, a total of 31 attacks I were observed in 15 patients. Six attacks were of grade 1, 18 of grade 2 and 7 of grade 3. General discomfort was reported in 8 attacks, reflex disorders were noted in 16, involvement of the musculature innervated by the cranial nerves in 2 and a positive Chvostek's sign in 12.

Fifteen *attacks II* were precipitated and allowed to abate spontaneously. In addition, at 9 tests various therapeutic and prophylactic measures were tried with prevention of the attack in 3 of them. Thus, a total of 21 attacks II were observed in 12 patients. Four attacks were of grade 1, 8 of grade 2 and 9 of grade 3. Soon after the administration of potassium some of the patients were troubled by nausea and mild epigastric pain. These symptoms abated before paralysis appeared. General discomfort when the attack reached its maximum was reported on 9 occasions, reflex disturbances on 15, involvement of the muscles innervated by the cranial nerves on 1 and a positive Chvostek's sign on 10.

Two *attacks III* were precipitated, both in the same patient. One attack was of grade 2, the other of grade 3. In the latter attack Chvostek's sign was positive. Reflex disorders were observed during both attacks.

Seven *attacks IV* were observed in 4 patients. In 3 (4 d, 4 e, 6 e) no laboratory studies were made. Therefore they are not included in Table 11, but only in Table 12 (see page 70). Five were of grade 2 and 2 of grade 3. General discomfort occurred in 2 attacks, reflex disturbances were recorded in 3, involvement of the musculature innervated by the cranial nerves in 1 and a positive Chvostek's sign in 2.

Blood Studies and Electrocardiographic Studies

Serum potassium. – For 1 of the 31 *attacks I* observed the culmination value is missing. In 30 attacks the serum potassium was 4.3–7.3 mEq/l at the culmination of the attack. In 14 of these attacks the values exceeded the normal upper limit given by Elliott & Holley (1951) as 5.5 mEq/l.

For 4 attacks the initial value is missing. In 22 of the remaining 26 attacks the serum potassium increased to reach its maximum within $\pm$ 15 minutes of the culmination of the attack. Abatement of the attack was accompanied by return of the serum potassium to the initial value or, as a rule, to a lower level. Exceptions were seen in only 4 attacks: 2 of grade 1 in one patient, and 2 of grade 2 in another. In 1 of these the culmination value was the same as, and in 3 attacks 0.1–0.6 mEq/l below, the initial value. Both of these patients belonged to type 2 with prolonged, less well defined attacks. The culmination value minus the initial value in these 26 attacks lay between -0.6 and $+2.4$ mEq/l, and was on the average 0.92 mEq/l (0.34 mEq/l for grade 1, 0.98 mEq/l for grade 2, and 1.32 mEq/l for grade 3).

For 1 attack the post-attack value is missing. In 18 attacks, which abated spontaneously (the others are not considered because the measures taken during those attacks might have influenced the post-attack values) both initial and post-attack values were determined. In 16 attacks the post-attack value was 0.2–1.3 mEq/l

below, and in 2 attacks 0.1–0.2 mEq/l above, the initial value. The post-attack value was on the average 0.43 mEq/l below the initial value (0 for grade 1, 0.38 for grade 2, and 0.90 for grade 3).

In 21 *attacks II* the culmination value was 4.8–7.1 mEq/l with only 3 values below 5.5 mEq/l and only 6 below 6.0 mEq/l (see page 100).

In all of the 21 attacks the serum potassium increased to reach a maximum within ± 15 minutes of the culmination of the attack. Abatement of the attack was accompanied by return of the serum potassium to roughly initial level. The culmination value minus the initial value in these 21 attacks lay between 0.4 and 2.6 mEq/l and was on the average 1.69 mEq/l (1.03 mEq/l for grade 1, 1.95 mEq/l for grade 2, and 1.76 mEq/l for grade 3).

In 14 attacks, which abated spontaneously, both initial and post-attack values were determined. The post-attack value was below the initial in 8 attacks, the same in 3, and above in 3.

In 2 *attacks III* the serum potassium increased and decreased with the development and the abatement of the attack. The culmination values noted were 6.6 mEq/l and 6.3 mEq/l, respectively, which were 2.2 mEq/l and 2.0 mEq/l higher than the initial values.

In 4 *attacks IV* the serum potassium was determined at the culmination of the attack. The values ranged between 6.0 mEq/l and 7.4 mEq/l.

Serum sodium. — The initial and culmination values were determined in 24 attacks I, 17 attacks II and 2 attacks III. The values found lay on the whole within the normal range of variation. The variations in the individual series were small and a slight increase occurred roughly as often as a slight decrease, *i. e.* no regular changes accompanied the attack.

Serum calcium and magnesium. — The serum calcium was determined in 5 attacks I, in 6 attacks II and in 1 attack III. The values found lay on the whole within the normal range of variation. The variations in the individual series were small and a slight increase occurred roughly as often as a slight decrease, *i. e.*, no regular changes accompanied the attack.

The serum magnesium values are not included in the table because only 2 such determinations were made, namely during an

attack I in patient No. 13 and during an attack II in patient No. 16. The initial values noted were 1.3 and 1.5 mEq/l, and the culmination values were 1.3 and 1.4 mEq/l. The values were thus somewhat low and did not change during attack.

Serum phosphorus. – The initial and culmination values were determined in 5 attacks I and 3 attacks II. All of the values found lay within normal limits for age. In 6 attacks the culmination value was slightly lower, and in 1 slightly higher, than the initial value, and in 1 the 2 values were the same.

Blood sugar. – The initial and culmination values were determined in 10 attacks I, 7 attacks II and in 1 attack III. The values found lay on the whole within the normal range of variation. The variations in the individual series were small, and a slight increase occurred roughly as often as a slight decrease, *i. e.*, no regular changes accompanied the attack.

Number of eosinophils. – Determinations were made before the physical exertion or the administration of potassium chloride, when the attack culminated, and every half hour for the next one hour and a half to 3 hours. The result of the examination was said to be positive when the number of eosinophils at culmination or afterwards was at least 50 per cent less than the original count. Only the initial value and the lowest subsequent value are given in the table. In 19 of 20 attacks I (the one in which the patient received an injection of ACTH not included), in 3 of 11 attacks II and in 1 attack III the result was positive. Of the factors possibly responsible for the decrease in the eosinophils, physical exertion is excluded as far as attacks II are concerned, as is to a certain extent the mental strain before and during the tests; when both kinds of attacks were precipitated in the same patient attack I was always precipitated before attack II.

The *electrocardiograms* were, as a rule, taken at the same time as the serum potassium was determined. During the attack the T-waves were higher and more pointed. These changes varied with the serum potassium. The changes were not so pronounced that they could be interpreted as pathologic on the basis of a single tracing. Therefore, electrocardiograms taken of the same patient every half hour during the test were compared. The result

was said to be positive when the aforementioned changes in the T-waves were seen transiently in the electrocardiograms made during the attack.

The heart frequency increased somewhat when the attack was at a maximum. No disturbances were seen in the rhythm, P–Q, QRS or Q–T.

Electrocardiograms were taken in 26 attacks I with a positive result in 18, in 19 attacks II with a positive result in 18, and in 2 attacks III with a positive result in both.

The electrocardiographic changes appeared at a serum potassium level varying from one examination to another. When the result was positive, the serum potassium at the culmination of the attack lay between 5.0 and 7.3 with a mean of 6.1 mEq/l; the culmination value minus the initial value, between 0.4 and 2.6 with a mean of 1.58 mEq/l. The corresponding figures for a negative result were 4.6 to 5.7 with a mean of 5.2 and -0.6 to $+1.4$ with a mean of 0.36.

A few *other blood determinations* were made, but are not included in the table. In all of these initial and culmination values were determined.

The serum chloride was determined in 3 attacks I, in 3 attacks II and in 1 attack III. The initial value was between 100 and 112 mEq/l; the culmination value, between 100 and 113 mEq/l. The culmination value minus the initial value was between -4 and $+3$ mEq/l.

The serum bicarbonate was determined in 3 attacks I, in 7 attacks II and in 1 attack III. The initial value lay between 20 and 29 mEq/l; the culmination value, between 21 and 29 mEq/l. The culmination value minus the initial value was between -5 and $+4$ mEq/l.

The hematocrit was determined in 12 attacks I, in 8 attacks II and in 1 attack III. The culmination value minus the initial value was between -2 and $+4$ per cent.

Results of Therapeutic and Prophylactic Measures

Table 12 summarises the observations made in tests in which prophylactic or therapeutic measures were tried and in those

Table 11. *Observations during attacks and provocation*

1. Patient's No.
2. Severity 0 = no attack 1 = mild 2 = moderate 3 = severe
3. Time (in min unless otherwise stated)
   T^1 = from end of exertion or potassium administration to onset of attack
   T^2 = from end of exertion or potassium administration to culmination
   T^3 = duration of attack
4. General discomfort
5. Reflex disorders
6. Involvement of muscles innervated by cranial nerves
7. Chvostek's sign
8. Serum potassium (mEq/l) a. initial value b. culmination value c. culmination minus initial value d. initial value minus post-attack value
9. Serum sodium (mEq/l) a. initial value b. culmination value
10. Serum calcium (mEq/l) a. initial value b. culmination value
11. Serum phosphorus (mg/100 ml) a. initial value b. culmination value
12. Number of eosinophils (per mm³) a. initial value b. culmination value
13. Blood sugar (g/100 ml) a. initial value b. culmination value
14. ECG changes
15. Remarks

1	2	3			4	5	6	7	8				9		10		11		12		13		14	15
		T^1	T^2	T^3					a	b	c	d	a	b	a	b	a	b	a	b	a	b		
No prophylactic or therapeutic measure																								
Attack I																								
1	2	15	60	105	—	—	—	—	5.3	7.3	2.0	0.4									0.09	0.08	+	Running up and down steps for 30 min
2a	2	30	60	105	—	—	—	—	4.9	6.4	1.5	0.7	140	134			6.4	6.4	176	6			+	Football: 2 hr
2b	2	30	75	75	—	—	—	—	4.7	6.0	1.3	0.6	141	139					313	13				Playing out-of-doors 1 hr
3a	3	25	45	70	—	+	—	—	5.5	6.8	1.3	1.2	141	139					100	13			+	Playing out-of-doors 1 hr 40 min
3b	2	30	75	80	—	—	—	—	4.0	5.2	1.2	0.2	140	139			5.8	5.6	47	0			+	Playing out-of-doors 1 hr
4a	2	40	55	80	—	+	—	—	3.9	5.8	1.9	0.4	135	140			5.0	4.5	231	44			+	Playing out-of-doors 1 hr 30 min
5a	2	50	60	45	+	+	—	—	5.1	5.7	0.6	0.6	142	138					231	19			+	Playing out-of-doors 1 hr 45 min
6a	2	60	110	>24 hr	—	—	—	+	4.0	5.7	1.7	0.3	138	138			5.0	3.8	244	81	0.10	0.11	+	Walking 1 hr 45 min
7a	1	30	60	>24 hr	+	—	—	—	4.9	5.7	0.8	0.3	147	140			3.7	3.9	531	0	0.11	0.11	+	Walking 2 hr
8a	3	30	90	120	+	+	—	+	4.4	6.8	2.4	0.4	142	136					197	6	0.10	0.11	+	Playing out-of-doors 1 hr 15 min
8b	2	30	50	90	—	—	—	—	4.2	6.0	1.8													Walking 2 hr
9a	2	30	55	20					1.6	5.0	0.4	0.3	140	140					656	81	0.09	0.08		Playing out-of-doors 1 hr 40 min
10a	2	75	90	60					1.7	5.6	0.9	0.2	140	141					175	81				Walking 2 hr
13a	2	90	125	>24 hr	[illegible]	[illegible]	[illegible]	[illegible]	1.1	5.4	1.0	0.4			5.3	5.4			213	56	0.07	0.11		Walking 1 hr
13b	3	45	90	>24 hr	[illegible]	[illegible]	[illegible]	[illegible]	4.6	5.8	1.2	1.1	139	137	4.6	4.6								Walking 1 hr 30 min
14a	3	30	75	>24 hr	[illegible]	[illegible]	[illegible]	[illegible]	4.6	5.8	1.2	0.9	142	140					138	13	0.09	0.10		Walking 1 hr 30 min

13a	2	90	125	>24 hr	—	+	—	+	4.4	5.4	1.0	0.4			5.3	5.4			213	56	0.07	0.11	+	Walking 1 hr
13b	3	45	90	>24 hr	+	+	+	+	4.6	5.8	1.2	1.1	139	137	4.6	4.6							+	Walking 1 hr 30 min
14a	3	30	75	>24 hr	+	+	+	+	4.6	5.8	1.2	0.9	142	140					138	13	0.09	0.10	+	Walking 1 hr 30 min
15a	2	30	75	195	+	—	—	+	4.1	5.4	1.3	0.1	140	142	4.4	4.4			181	100	0.11	0.09	+	Walking 1 hr 30 min
17a	1	45	60	60	—	—	—	—	4.2	5.2	1.0	−0.2	148	146	5.5	5.4							—	Walking 1 hr 30 min
Attack II																								
4c	3	20	45	75	+	+	—	—	4.8	6.5	1.7	0.6	142	141					75	69	0.11	0.10	+	3 g KCl
6c	3	75	105	150	+	+	—	+	4.3	6.7	2.4	−0.2					3.9	3.5	119	106			+	4 g KCl
8d	3	45	60	60	+	+	—	+	5.0	6.8	1.8	0.4	144	142					44	37			+	3 g KCl
9b	2	5	30	70	—	—	—	—	5.3	7.1	1.8	0.3	142	140					643	41			+	2 g KCl
10c	3	30	70	160	+	+	+	—	4.7	6.1	1.4	0.2	146	147							0.11	0.09	+	4 g KCl
11c	2	20	75	145	—	+	—	+	4.2	6.7	2.5	0.2							206	69	0.10	0.12	+	4 g KCl
12a	1	50	60	40	—	—	—	—	4.3	5.7	1.4	0.0	138	138									—	4 g KCl
13f	2	30	75	150	—	+	—	+	4.4	5.3	0.9	0.0											+	4 g KCl
13g	3	30	60	>24 hr	—	+	—	+	5.4	5.8	0.4	1.0			5.3	5.3			320	250	0.06	0.11	+	5 g KCl
14b	2	30	105	225	+	+	—	+	3.9	6.0	2.1	0.2	136	140	5.6	5.3			25	19			+	4 g KCl
15d	3	40	100	180	+	+	—	+	4.5	6.7	2.2	0.3	141	143	4.5	4.6			191	181			+	4 g KCl
15e	3	30	110	180	+	+	—	+	4.4	6.3	1.9		138	138									+	4 g KCl
16a	2	45	85	135	—	+	—	+	4.6	7.1	2.5	−1.1	144	140	4.3	4.1	4.2	3.1	56	113	0.10	0.09	+	5 g KCl
17b	1	45	55	60	—	—	—	—	4.8	6.2	1.4	0.0	140	140			4.8	4.2	38	13	0.10	0.09	+	4 g KCl
17c	2	70	85	80	—	—	—	—	4.5	7.1	2.6	−0.4	143	140	5.5	5.4							+	5 g KCl
Attack III																								
12b	2	45	60	195	—	+	—	—	4.4	6.6	2.2	0.3	142	139					100	19	0.10	0.10	+	Walking 2 hr 4 g KCl
Attack IV																								
5b	2			30	+	+	—	—		7.4														
Effect of calcium i.v.																								
Attack I																								
6b	2	45	75	70	—	—	—	—	4.6	5.2	0.6	1.2	130	134									+	Walking 2 hr
7b	1	0	20	50	—	—	—	—	5.2	4.6	−0.6	0.2	132	140					350	125			—	Walking 2 hr
8c	3	10	20	60	+	+	—	+		7.3									125	43				Running up and down steps for 50 min
13c	2	60	105	65	—	—	—	+	4.4	4.3	−0.1	0.4	138	142										Walking 1 hr
15b	3	60	100	55	+	+	—	+	4.9	5.4	0.5	0.9	140	138	4.9	4.6							—	Walking 1 hr 30 min

1	2	3 T¹	T²	T³	4	5	6	7	8 a	b	c	d	9 a	b	10 a	b	11 a	b	12 a	b	13 a	b	14	15
Attack II																								
10d	3	30	70	100	+	+	—	—	4.5	6.0	1.5	–0.1	150	154					125	88	0.11	0.10	+	4 g KCl
13h	2		90	>24 hr	—	+	—	+	4.0	5.4	1.4	–0.7	146	147										4 g KCl
16b	3	40	70	50	+	+	—	—	4.5	7.0	2.5	–0.2	139	142	5.0	4.9							+	5 g KCl
Attack III																								
12c	3	45	65	45	—	+	—	+	4.3	6.3	2.0	–0.1	138	137	5.1	5.5							+	Walking 2 hr 5 g KCl
Attack IV																								
6d	3			60	+	—	+	+		6.5														
Effect of glucose with or without insulin																								
Attack I																								
4b	3	20	30	60	—	+	—	—		5.4									331	156			+	Playing out-of-doors 1 hr 30 min
10b	2	60	80	60	—	+	—	—	5.2	5.4	0.2	0.9	146	148									—	Walking 1 hr 10 min
13d	2		75	>24 hr	—	+	—	+	5.2	4.6	–0.6	0.6	146	152									—	Walking 1 hr
Provocation II																								
10e	1	50	60	30	—	—	—	—	4.1	4.8	0.7		141	141										4 g KCl
10f	0								4.5	5.8	1.3	0.0	148	150										4 g KCl
11d	2	30	60	>24 hr	—	+	—	—	4.9	6.7	1.8	0.9	144	145									+	5 g KCl
11e	1	15	30	75	—	—	—	—	5.2	5.8	0.6	0.9	143	138									+	4 g KCl
15f	0								3.8	5.4	1.6	–0.7	146	146										4 g KCl
16c	0								4.4	4.8	0.4	–0.2	134	136										5 g KCl
Effect of Ringer's solution (6f, 7c, 15c), ACTH (13e) and prostigmine (8e)																								
Attack I																								
7c	1	0	30	150	—	—	—	—	5.0	5.0	0.0	0.3	138	142					144	19			—	Walking 2 hr
13e	2	60	90	>24 hr	—	+	—	+		4.5									413	213				Walking 1 hr
15c	2	30	90	165	—	+	—	+															+	Walking 1 hr 30 min
Attack IV																								
6f	2	15	75	105						6.4														

which where allowed to abate spontaneously. The attacks are designated in the same way as in Table 11, where the results of the laboratory studies are given (except for 3 attacks IV, 4 d, 4 e, 6 e, where no laboratory studies were made). Since the time between the culmination of the attack and its complete abatement was taken for evaluating a prophylactic or therapeutic measure in a given attack, this time is included in Table 12. Unless otherwise stated, the time is given in minutes. The effect was judged as good when the attack lasted only at most half as long as that observed for spontaneous recovery, by the same patient, from an attack of roughly equal severity and, if possible, precipitated in the same way.

Every patient was thus compared with himself and, with but one exception, the comparison was made between attacks observed in hospital. The duration of the attacks could vary widely in one and the same patient. Thus a patient who had attacks of more than 24 hours' duration also and, as a rule, more frequently had attacks that abated completely within a few hours. The symptoms persisting for more than a few hours after the culmination of a prolonged attack are peculiar: slight weakness, dull pain, stiffness and muscle tenderness. Such prolonged attacks where no prophylactic or therapeutic measures were taken were therefore not used for comparison in the evaluation of the effect of such measures. In 1 patient (No. 7) only 1 attack for which he received neither prophylaxis nor therapy was observed, and it lasted more than 24 hours. Therefore, in contrast to the other patients, in him, the effect of therapeutic measures must be judged by comparison between the attacks observed in hospital and his statement of the time an attack of roughly the same severity usually required to disappear when he did not try to accelerate the course by eating or moving about. (This information is also given in Table 12.)

Calcium. — Calcium "Sandoz" 10 per cent (calcium gluconate) was administered intravenously in a dose of 5–20 ml.

In an attack I the injection was given about 15 minutes after the attack had culminated. The injection might possibly have accelerated the course somewhat, but the effect was difficult to judge because the paresis was already abating. When administered

Table 12. *Effect of prophylactic and therapeutic measures*

Patient's No.	Attack or provocation	Severity 0 = no attack 1 = mild 2 = moderate 3 = severe	Measures	Interval between culmination and end of attack (in min unless otherwise stated)
4a	I	2	None	65
4b	I	3	90 g glucose orally + 20 I.U. insulin s.c. at culmination	50
4d	IV	2	5 ml 10 % calcium gluconate i.v. at culmination	5
4e	IV	3	10 ml 10 % calcium gluconate i.v. at culmination	15
6a	I	2	None	> 24 hr
6b	I	2	10 ml 10 % calcium gluconate i.v. 15 min after culmination	40
6c	II (4 g KCl)	3	None	120
6d	IV	3	20 ml 10 % calcium gluconate i.v. at culmination	45
6e	IV	2	15 ml 10 % calcium gluconate i.v. at culmination	15
6f	IV	2	20 ml Ringer's solution i.v. at culmination	75
7a	I	1	None	> 24 hr
7b	I	1	20 ml 10 % calcium gluconate i.v. at culmination	30
7c	I	1	20 ml Ringer's solution i.v. at culmination	120
(7	IV		None	1—2 hr)[1]
8a	I	3	None	60
8b	I	2	None	70
8c	I	3	10 ml 10 % calcium gluconate i.v. at culmination	50
8e	IV	2	1 mg prostigmine + 0.5 mg atropine i.v. at culmination	55
10a	I	2	None	45
10b	I	2	50 ml 50 % glucose i.v. at culmination	40

[1] According to patient's report. Attack not observed in hospital.

10c	II (4 g KCl)	3	None	120
10d	II (4 g KCl)	3	20 ml 10 % calcium gluconate i.v. at culmination	60
10e	II (4 g KCl)	1	150 g glucose orally + KCl simultaneously	20
10f	II (4 g KCl)	0	150 g glucose orally + 20 I.U. insulin s.c. 25 min before KCl	
11c	II (4 g KCl)	2	None	90
11d	II (5 g KCl)	2	50 ml 50 % glucose i.v. + 20 I.U. insulin i.v. at culmination	>24 hr
11e	II (4 g KCl)	1	100 g glucose orally + 20 I.U. insulin s.c. 25 min before KCl	60
12b	III (4 g KCl)	2	None	180
12c	III (5 g KCl)	3	20 ml 10 % calcium gluconate i.v. at culmination	25
13a	I	2	None	>24 hr
13b	I	3	None	>24 hr
13c	I	2	20 ml 10 % calcium gluconate i.v. at culmination	20
13d	I	2	50 ml 50 % glucose i.v. at culmination	>24 hr
13e	I	2	25 mg ACTH i.m. at culmination	>24 hr
13f	II (4 g KCl)	2	None	105
13g	II (5 g KCl)	3	None	>24 hr
13h	II (4 g KCl)	2	20 ml 10 % calcium gluconate i.v. 2 hr after culmination	>24 hr
15a	I	2	None	150
15b	I	3	20 ml 10 % calcium gluconate i.v. at culmination	15
15c	I	2	20 ml Ringer's solution i.v. at culmination	105
15d	II (4 g KCl)	3	None	120
15e	II (4 g KCl)	3	None	100
15f	II (4 g KCl)	0	100 g glucose orally 30 min before KCl	
16a	II (5 g KCl)	2	None	95
16b	II (5 g KCl)	3	20 ml 10 % calcium gluconate at culmination	20
16c	II (5 g KCl)	0	100 g glucose orally 30 min before KCl	

at culmination the injection had a beneficial effect in 3 attacks I, in 2 attacks II, in 1 attack III and in 4 attacks IV, but no effect in 1 attack I. Neither did it produce any demonstrable effect when administered several hours after the paralysis had reached its maximum during a prolonged attack II.

Glucose and insulin. — Glucose, with or without insulin, administered orally or intravenously was tried in 3 attacks I and in 1 attack II at the culmination of the attack, but without effect.

In 1 case glucose was tried at the same time as potassium. This produced a short mild attack. An equal dose of potassium had earlier precipitated a grade 3 attack in that patient.

Administration of glucose about half an hour before the administration of potassium: 4 tests were carried out, 2 with and 2 without simultaneous administration of insulin. No attack occurred in 3, and a mild attack in 1; in these 4 patients an equal dose of potassium had earlier precipitated grade 2 or 3 attacks.

Ringer's solution. — In order to avoid the psychologic effect of the injection, 3 of the patients in whom calcium had had a beneficial effect received Ringer's solution intravenously at the culmination of another attack of roughly the same severity, in the belief that it was calcium, but in none of them did the injection produce any demonstrable effect.

Prostigmine and ACTH. — Prostigmine intravenously was tried on one patient and ACTH intramuscularly on another at the culmination of the attack, but without any demonstrable effect in either.

Urinalysis

The colour of the urine did not change during attacks.

Heller's test done during 6 attacks in 2 patients, urobilinogen and benzidine tests in 20 attacks in 5 patients regularly gave a negative result. The excretion of creatine and creatinine was determined in 2 patients for 1 day without an attack and for 1 day with an attack. All of the values were normal and no change occurred in connection with the attack.

The excretion of potassium during an attack and during a free interval is accounted for in Table 13. As mentioned in Chapter IV,

Table 13. *Urinary potassium (mEq/hr) in patients.*

Patient's No.	During free interval	Attack I	Attack II	Attack III	Remarks
6	1.2	8.1			
7		9.6			Received 20 ml Ringer's solution
8	0.7	4.9	2.4		
		4.9			
9	1.1	1.0	6.9		
	1.9				
10	2.9	4.4	8.3		
	1.5				
11	4.1	6.4	7.4		
	2.9				
12			9.8	9.2	
13	1.6	4.0	6.5		
	1.9	2.6	5.5		
	2.9				
	9.2				
14		13.3	4.6		
15	0.9	8.7	16.2		
	1.1	6.4	13.7		Received 20 ml Ringer's solution during I
16	2.9		7.2		
	1.9				
17	3.4	4.3	2.0		
	3.2				

samples were collected at about the same hour on attack-free days as on those days attacks were precipitated.

The patient voided the bladder at the end of the physical exertion or immediately before the administration of potassium and after the attack. Sometimes the patient could not pass water at the time desired and then it was necessary to postpone collection for at most half an hour. In the attacks that lasted more than a day the sample was collected a few hours after the attack had reached a maximum. The period for which the excretion was measured thus included – with the aforementioned reservations – a period immediately before the attack and the actual attack.

During a *free interval* the excretion of potassium in the urine in 9 patients lay between 0.7 and 9.2 mEq/hour with a mean of 2.5 mEq/hour.

In 13 *attacks I* the excretion of potassium lay between 1.0 and 13.3 mEq/hour with a mean of 6.0 mEq/hour.

In 12 *attacks II* the excretion of potassium lay between 2.0 and 16.2 mEq/hour with a mean of 7.5 mEq/hour.

IN SUMMARY:

1) Seventeen patients were observed during altogether 61 attacks, 10 of grade 1, 32 of grade 2 and 19 of grade 3. General discomfort was noted in 19 attacks, reflex disorders in 36, involvement of the musculature innervated by the cranial nerves in 4 and a positive Chvostek's sign in 25.

2) In 45 of 49 attacks the serum potassium increased and decreased with the development and the abatement of the attack. In 39 of 57 (including 18 of 34 in which the patients received no potassium) the serum potassium increased to more than 5.5 mEq/l. In 16 of 18 attacks in which the patients received no potassium the post-attack value was lower than the initial.

3) In 6 of 8 attacks the serum phosphorus decreased slightly, but never fell below the normal lower limit.

4) In 23 of 32 attacks the number of eosinophils decreased by at least 50 per cent of the initial count. The patients can thus react with a decrease in the number of eosinophils to the stress to which they are subjected by the actual attack, the physical exertion and the mental strain in association with the test.

5) In 38 of 47 attacks studied electrocardiographically transient changes were noted in the shape and height of the T-waves. In every individual attack the electrocardiographic changes coincided in time with the variations in the serum potassium.

6) No connection was found between the attack and the serum sodium (43 attacks studied), serum calcium (12 attacks studied), serum magnesium (2 attacks studied), blood sugar (18 attacks studied), serum chloride (7 attacks studied), serum bicarbonate (11 attacks studied) or hematocrit (21 attacks studied).

7) Calcium administered intravenously at culmination of 11 attacks produced a good effect in 10, administered intravenously about 2 hours after the culmination of 1 attack it produced no definite effect.

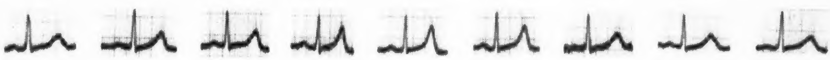
8) The administration of glucose with or without insulin was tried at the culmination of 4 attacks without any beneficial effect. When administered simultaneously with, or about half an hour before, an otherwise provocative dose of potassium, no attack occurred in 3 tests out of 5 and a mild attack in the remaining 2.

9) No demonstrable effect was produced by Ringer's solution intravenously in 3 attacks, by prostigmine intravenously in 1 or by ACTH intramuscularly in 1. The injections were regularly given at the culmination of the attack.

10) The urinary excretion of potassium was not low during a free interval and did not decrease during an attack.

The findings in 8 attacks are given in detail below:

Patient No. 7 (male, aged 20). Attack I a. Severity: Grade 1

lour	7.30	9.30	10.00	10.30	11.00	11.30	12.00	12.30	13.00
CG ead II									
erum otassium nEq/l)	4.9	4.9	5.0	5.7	4.9	5.0	4.7	4.7	4.6

emarks Walking 7.30–9.30. Onset of attack 10.00. Culmination 10.30. End of attack about 3 p. m. next day.

The test was carried out on December 9, 1954. Before the test the gross functional strength was normal, the reflexes were normal and Chvostek's sign was negative. Dynamometer recordings: right 110, left 60. Electric excitability, as tested on the fibular nerve, was normal: faradic current produced normal tetanic contraction, galvanic current produced CCC at 2.6 and ACC at 4.0 milliamperes.

The patient was in the fasting state throughout the test.

7.30 a. m. – The patient was sent out for a walk.

9.30 a. m. – The patient returned and lay down on the examination table. No symptoms.

10.00 a. m. – Feeling of heaviness in the extremities and decreased hand strength. Dynamometer recordings: right 40, left 25. No definite weakness of the legs.

10.30 a. m. – Paralysis reached a maximum. The patient could still move about on the table, sit up, stand and walk. However, he could only walk slowly and with difficulty. Both dorsal and plantar flexion of the feet were weaker, but otherwise no weakness of the lower extremities was noted. The musculature innervated by the cranial nerves was not involved. The tendon reflexes were as before the attack.

Electric excitability, as tested on the median nerve: faradic current produced normal tetanic contraction, galvanic current produced CCC at 2.3 and ACC at 4.0 milliamperes. Percussion of the thenar muscles produced no myotonic contraction. Chvostek's sign was negative.

Consciousness was preserved. The skin was cold and clammy, and the patient complained of nausea, unrest and palpitation. Respiration was normal.

Superficial and deep sensibility was normal.

11.00 a. m. – The patient now felt better. Walking was no longer difficult and no weakness of the feet was demonstrable. The arms were still weak.

12.00 noon. – Continued gradual improvement.

3.00 p. m. – The arms had recovered normal strength, but the hands were still weak. Dynamometer recordings: right 50, left 15. Extension of the fingers was somewhat decreased, especially of the little finger. Otherwise no signs of weakness were noted. The reflexes were normal.

3.00 p. m. on the following day. – The patient had now completely recovered.

Patient No. 4 (female, aged 10). Attack I a. Severity: Grade 2

Hour	7.00	9.15	9.45	10.15	10.45	11.15	11.45	12.15
ECG Lead II								
Serum potassium (mEq/l)	3.9	4.0	3.9	5,8	4,6	4.1	3.5	3.5
Remarks	Playing out-of-doors 7.15—9.15. Onset of attack 9.55. Culmination 10.10. End of attack 11.15.							

The test was carried out on August 19th, 1954. Before the test the gross functional strength was normal. The reflexes were normal and Chvostek's sign was negative. Electric excitability, as tested on the fibular nerve, was normal: faradic current produced normal tetanic contraction, galvanic current produced CCC at 2.0 milliamperes.

The patient was in the fasting state throughout the test.

7.45 a. m. – The patient was sent out to play.

9.15 a. m. – The patient returned and lay down. No symptoms.

9.55 a. m. – Symptoms began to appear in the form of a feeling of heaviness of the extremities. No definite weakness.

10.00 a. m. – There were now slight weakness of the legs and decreased hand strength.

10.10 a. m. – The attack reached a maximum. The patient could lift the head from the pillow and turn over on the examination table, but could not sit up. She could clench the hands and stretch the fingers completely, but the hand strength was impaired. She could lift the

arms, but not against resistance. She could not lift the legs. When the legs were passively lifted, she could not hold them up; they fell down heavily onto the table. She could move the feet but the strength of the dorsal flexion was definitely decreased. The musculature innervated by the cranial nerves was not involved. The patellar and Achilles tendon reflexes were weaker bilaterally than before the attack. No other reflex disorders were noted. Electric excitability, as tested on the fibular nerve: faradic current produced normal tetanic contraction, galvanic produced CCC at 2.0 milliamperes. Percussion of the thenar muscles produced no myotonic contraction. Chvostek's sign was negative.

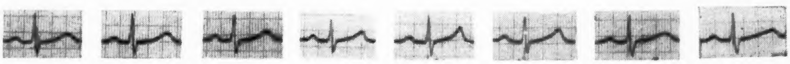
Consciousness was preserved and the patient stated that she had no general discomfort. Respiration was normal.

Superficial and deep sensibility was normal.

10.30 a. m. – The strength of the arms and hands was now practically normal. The strength of the feet was better, but she was still unable to lift the legs from the table.

11.15 a. m. – The patient had now recovered completely.

Patient No. 8 (male, aged 10). Attack I a. Severity: Grade 3

Hour	7.00	9.00	9.30	10.00	10.30	11.00	11.30	12.00
ECG Lead II								
Serum potassium mEq/l	4.4	4.5	5.4	6.0	6.8	5.7	5.0	4.0
Remarks	Playing out-of-doors 7.45—9.00. Onset of attack 9.30. Culmination 10.30. End of attack 11.30.							

The test was carried out on January 10th, 1955. Before the test the gross functional strength was normal, the reflexes were normal and Chvostek's sign was negative. Dynamometer recordings: right 70, left 65. Electric excitability, as tested on the fibular nerve, was normal: faradic current produced normal tetanic contraction, galvanic current produced CCC at 2.0 and ACC at 2.5 milliamperes.

The patient was in the fasting state throughout the test.

7.45 a. m. – The patient was sent out to play.

9.00 a. m. – The patient returned and lay down. No symptoms.

9.30 a. m. – The patient complained of a feeling of heaviness of the legs, and the strength of the legs and hands was now decreased. He could lift the extended legs from the table, but only against at most slight resistance. The hand strength was decreased, dynamometer recordings: right 40, left 40.

10.30 a. m. – The paralysis reached a maximum. The patient could not turn on the examination table, he could not sit up and he could lift

the head from the pillow only with difficulty. He could not lift the extremities. He could still move the hands but not the feet on the table.

When an extremity was passively lifted, the patient could not hold it up; it fell down heavily onto the table. He could clench the hands and extend the fingers completely. Dynamometer recordings: right 15, left 5. The musculature innervated by the cranial nerves was not involved. All of the tendon reflexes were present and equal bilaterally, but were weaker than before the attack, the other reflexes were normal. Electric excitability, as tested on the fibular nerve: faradic current produced normal tetanic contraction, galvanic current produced CCC at 2.0 and ACC at 2.5 milliamperes. Percussion of the thenar muscles produced no myotonic contraction. Chvostek's sign was positive.

The patient was depressed and complained of nausea. There was no cold sweat. Consciousness was preserved. Respiration was normal.

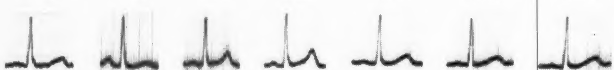
Superficial and deep sensibility was normal.

10.50 a. m. – The patient could now lift the head from the pillow and turn over on the examination table.

11.15 a. m. – He could now sit up, stand, and he began to walk with support. He could lift the extended legs against slight resistance. The hand strength was better: dynamometer recordings: right 40, left 45.

11.30 a. m. – The patient had now completely recovered.

Patient No. 17 (male, aged 31). Attack II b. Severity: Grade 1

Hour	7.00	8.00	8.30	9.00	9.30	10.00	10.30
ECG Lead II							
Serum potassium (mEq/l)	4.8	5.1	6.2	5.6	5.6	5.2	4.8

Remarks 4 g KCl 7.45. Onset of attack 8.30. Culmination 8.40. End of attack 9.30.

The test was carried out on October 18th, 1954. Before the test the gross functional strength was normal, the reflexes were normal and Chvostek's sign was negative. Dynamometer recordings: right 120, left 90. Electric excitability, as tested on the fibular nerve, was normal: faradic current produced normal tetanic contraction, galvanic current gave CCC at 2.0 and ACC at 3.4 milliamperes.

The patient was in the fasting state throughout the test.

7.45 a. m. – Oral administration of 4 g potassium chloride in water solution and a glass of water.

8.30 a. m. – Initial symptoms in the form of a feeling of heaviness of the legs.

8.40 a. m. – The paralysis reached a maximum. The patient could sit up, stand and walk, but only slowly and with difficulty. His legs were weak and although he could lift them from the bed, it was only against less resistance than before the attack. Dorsal flexion of the foot was also weaker than before the attack. The entire right leg and foot were weaker than the left. The gross functional strength of the hand also decreased; dynamometer recordings: right 90, left 80. The musculature innervated by the cranial nerves was not involved. The reflexes were normal, equal on both sides, and the same as before the attack. Electric excitability, as tested on the fibular nerve: faradic current gave normal tetanic contraction, galvanic current produced CCC at 2.0 and ACC at 3.4 milliamperes. Percussion of the thenar muscles produced no myotonic contraction. Chvostek's sign was negative.

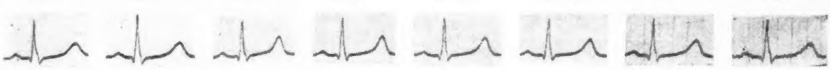
Consciousness was preserved and the patient stated that he felt no general discomfort. Respiration was normal.

Superficial and deep sensibility was normal.

9.00 a. m. – Strength of the arms and hands was now as before the attack, *i. e.*, dynamometer: right 120, left 90. There was still slight weakness of the right leg.

9.30 a. m. – The patient had now completely recovered.

Patient No. 11 (male, aged 16). Attack II c. Severity: Grade 2

ur	7.30	8.30	9.00	9.30	10.00	10.30	11.00	11.30
G, d II								
um ssium Eq/l)	4.2	4.8	5.7	6.7	5.2	5.1	4.0	—
arks	4 g KCl 8.10. Onset of attack 8.30. Culmination 9.25. End of attack 10.55.							

The test was carried out on February 28th, 1955. Before the test the gross functional strength was normal, the reflexes were normal and Chvostek's sign was negative. Dynamometer recordings: right 100, left 100. Electric excitability, as tested on the fibular nerve, was normal: faradic current produced normal tetanic contraction, galvanic current produced CCC at 1.5 and ACC at 2.0 milliamperes.

The patient was in the fasting state throughout the test.

8.10 a. m. – Oral administration of 4 g potassium chloride in water solution and a glass of water.

8.30 a. m. – Initial symptoms in the form of fatigue and clumsiness of the hands. Dynamometer recordings: right 90, left 60.

9.00 a. m. – Paralysis progressed. Now also weakness of the legs.

9.25 a. m. – Paralysis reached a maximum. The patient could turn on the examination table and lift the head from the pillow but could

not sit up. He could lift the arms but not the legs. He could move the feet on the table. Dorsal flexion of the feet was also weak. When the legs were lifted passively, he could hold them up for a short time. He could clench the hands and extend the fingers completely. Dynamometer recordings: right 30, left 15. The musculature innervated by the cranial nerves was not involved. The Achilles tendon reflexes were absent, the other muscle reflexes, which were equal on both sides, were weaker than before the attack. The pupillary, abdominal and plantar reflexes were normal. Electric excitability, as tested on the fibular nerve: faradic current produced normal tetanic contraction, galvanic current produced CCC at 1.5 and ACC at 2.0 milliamperes. Percussion of the thenar muscles did not produce myotonic contraction. Chvostek's sign was positive.

Consciousness was preserved and he stated that he had no general discomfort. Respiration was normal.

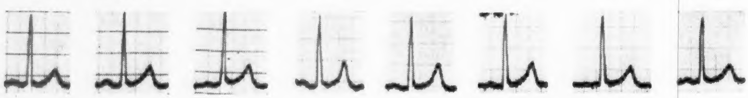
Superficial and deep sensibility was normal.

9.50 a. m. – The arms and the hands began to recover strength but not the legs.

10.30 a. m. – Now the legs also began to recover strength. He could now lift the legs against slight resistance.

10.55 a. m. – The patient had now completely recovered.

Patient No. 6 (female, aged 28). Attack II c. Severity: Grade 3

Hour	7.15	8.45	9.15	9.45	10.15	10.45	11.15	11.45	12.1
ECG Lead II									
Serum potassium (mEq/l)	4.3	4.8	5.9	6.7	6.1	5.3	4.3	4.2	4.
Remarks	4 g KCl 8.15. Onset of attack 9.30. Culmination 10.00. End of attack 12.00.								

The test was carried out on September 27th, 1954. Before the test the gross functional strength was normal, the reflexes were normal and Chvostek's sign was negative. Electric excitability, as tested on the fibular nerve, was normal: faradic current produced normal tetanic contraction, galvanic current produced CCC at 2.5 milliamperes.

The patient was in the fasting state throughout the test.

8.15 a. m. – Oral administration of 4 g potassium chloride in water solution and a glass of water.

9.30 a. m. – The arms and legs were now demonstrably weaker. The patient could lift the legs from the bed but against less resistance than before the attack. Hand strength was decreased.

10.00 a. m. – The attack reached a maximum. The patient could

lift the head from the pillow but she could not turn over on the examination table, nor could she sit up. She could move the fingers but she could not clench her hands or completely extend the fingers, she could not move the feet or toes at all. When an extremity was passively lifted the patient could not hold it up, it fell heavily onto the table. The musculature innervated by the cranial nerves was not involved. The patellar reflex was somewhat weaker bilaterally than before the attack, but the other reflexes were normal. Electric excitability, as tested on the fibular nerve: faradic current produced normal tetanic contraction, galvanic current produced CCC at 2.8 milliamperes. Percussion of the thenar muscles produced no myotonic contraction. Chvostek's sign was positive.

Consciousness was preserved. She felt depressed and was troubled by palpitations. Respiration was normal.

Superficial and deep sensibility was normal.

10.15 a. m. – Slight improvement subjectively but not objectively.

10.45 a. m. – The patient was now able to move about at will on the examination table, she could turn over, but she could sit up only with difficulty. She could lift the legs but not against resistance.

11.00 a. m. – The patient could now lift the extended legs even against slight resistance. There was no definite weakness of the arms or hands. She could stand and walk slowly with difficulty. The reflexes were normal. Chvostek's sign was positive.

12.00 noon. – The patient had now completely recovered.

Patient No. 15 (female, aged 20). Attack I b. Severity: Grade 3
Effect of calcium intravenously

Hour	7.00	8.45	10.10	10.50
ECG Lead II				
Serum potassium (mEq/l)	4.9	4.6	5.4	4.0
Serum calcium (mEq/l)	4.9		4.6	5.2

Remarks Walking 7.00–8.30. Onset of attack 9.30. Culmination, calcium i.v. 10.10–10.15. End of attack 10.25.

The test was carried out on June 3, 1955. Before the test the gross functional strength was normal, the reflexes were normal and Chvostek's sign was negative.

The patient was in the fasting state throughout the test.

7.00 a. m. – The patient was sent out for a walk.

8.30 a. m. – The patient returned and lay down. No symptoms.

9.30 a. m. – Initial symptoms in the form of fatigue and feeling of heaviness of the extremities, slight weakness of the legs and arms but normal hand strength and normal strength of dorsal flexion of the foot. The reflexes were normal. Chvostek's sign was negative.

9.50 a. m. – The paralysis progressed. The patient could now lift the legs against gravity, but not against any resistance. Hand strength was decreased.

10.10–10.15 a. m. – Paralysis reached a maximum. The patient could not turn over on the examination table, she could not lift the head from the pillow or sit up, she could not lift the extended legs from the table. She could lift the hands but not the arms. She could clench the hands and extend the fingers completely, but the hands were weak. The strength of the feet was only slightly impaired. When an extremity was passively lifted, the patient could hold it up for a short time, after which it fell heavily onto the table. The musculature innervated by the cranial nerves was not involved. Pupillary, abdominal and plantar reflexes were normal, the auto-reflexes of the arms, patellar and Achilles tendon reflexes were demonstrable equal bilaterally, but were weaker than before the attack. Percussion of the thenar muscles produced no myotonic contraction. Chvostek's sign was positive.

Consciousness was preserved. The skin was clammy and cold and the patient complained of general, diffuse discomfort. Respiration was normal.

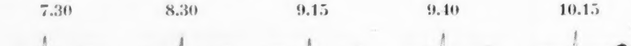
Superficial and deep sensibility was normal.

10.10–10.15 a. m. – The patient received 20 ml calcium "Sandoz" 10 per cent (calcium gluconate) intravenously.

10.20 a. m. – Rapid improvement. The patient could now turn over on the examination table, lift the head from the pillow and sit up, though with difficulty. The strength of the arms and hands was normal. The patient could lift the extended legs but not against resistance. The reflexes were normal and Chvostek's sign was negative.

10.25 a. m. – The patient had now completely recovered.

Patient No. 16 (female, aged 37). Attack II b. Severity: Grade 3
Effect of calcium intravenously

Hour	7.30	8.30	9.15	9.40	10.15
ECG Lead II					
Serum potassium (mEq/l)	4.5	6.3	7.0	4.8	4.7
Serum calcium (mEq/l)	5.0		4.9	5.1	
Remarks	5 g KCl 8.00. Onset of attack 8.40. Culmination, calcium i. v. 9.10—9.15. End of attack 9.30.				

The test was carried out on June 20th, 1955. Before the test gross functional strength was normal, reflexes were normal and Chvostek's sign was negative.

The patient was in the fasting state throughout the test.

8.00 a. m. – Oral administration of 5 g potassium chloride (in the form of uncoated tablets) and a glass of water.

8.40 a. m. – Initial symptoms in the form of pins and needles and a feeling of stiffness of the face and of heaviness of the extremities. No confirmatory signs noted.

8.50 a. m. – Decreased strength was now demonstrable. The patient could lift the extended legs but not against resistance. There was distinct weakness of the arms and legs. The reflexes were not changed with certainty.

9.10–9.15 a. m. – The attack reached a maximum. The patient could not turn on the examination table, she could not lift the head from the pillow and could not sit up. She could move the hands and feet on the table, but she could not lift the extremities. When an extremity was passively lifted, she could not hold it up; it fell heavily onto the table. She could clench the hands but she could not hold anything with them, and she could not stretch the fingers completely. She could move the feet and toes, but not against resistance. The musculature innervated by the cranial nerves was not involved. Pupillary, abdominal and plantar reflexes were normal. The autoreflexes of the arms and the patellar reflexes were weaker than before the attack. The Achilles tendon reflexes were absent. Percussion of the thenar muscles produced no myotonic contraction. Chvostek's sign was negative.

Consciousness was preserved. The patient complained of general discomfort. Respiration was normal.

Superficial and deep sensibility was normal.

9.15 a. m. – The patient received 20 ml calcium "Sandoz" 10 per cent (calcium gluconate) intravenously.

9.20 a. m. – Some improvement in the strength of the arms and hands was noted. The patient could now also lift the head from the pillow.

9.25 a. m. – Rapid improvement. The strength of the arms and hands was now normal. The patient could lift the legs, but not against resistance.

9.30 a. m. – The patient had now completely recovered.

Examination of the Cerebrospinal Fluid

Examination of cerebrospinal fluid obtained by lumbar puncture of patient No. 8 during the maximum phase of attack I b showed

no signs of a pathologic condition. The potassium content was 3.4 mEq/l and the sodium content was 148 mEq/l (normal: 2.50–3.65 and 134–152 mEq/l, respectively, SHAW & HOLLEY 1951).

Electroencephalography *

Electroencephalograms were taken of patient No. 1 before and during an attack IV with the same normal result on both occasions.

Electromyographic Findings and the Effect of Acetylcholine Injected Intra-arterially

Electromyography. – The left biceps brachii of four patients (Nos. 8, 12, 13 and 16)** were studied electromyographically. In three of them the examination was carried out both before and during a fully developed attack of paresis. In the fourth (No. 13) the examination was carried out during severe paresis as well as later when the attack was abating. All patients were studied for the pattern of the action potentials during attempt at maximum innervation, for any activity in the relaxed muscle, for the mean duration of the action potentials and for the occurrence of polyphasic potentials. The activity pattern of maximum contraction has been described by BUCHTHAL & CLEMMESEN (1941) and BUCHTHAL & PINELLI (1952). Readers interested in the technique and criteria for assessing the duration of the action potentials are referred to BUCHTHAL, GULD & ROSENFALCK (1954). The means for a normal material have been taken from BUCHTHAL & ROSENFALCK (1955). In addition to these examinations which belong to routine electromyography, in 3 of the 4 patients the velocity was determined at which an action potential elicited by electrical stimulation is propagated over the muscle (BUCHTHAL, GULD & ROSENFALCK 1955 a and b).

The electromyographic results are given in Table 14. *During*

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* The electroencephalographic studies were carried out at the Department of Neurology, University of Lund.

** The electromyographic studies were carried out at the Institute of Neurophysiology, University of Copenhagen, and the tracings were analysed by Professor Fritz Buchthal.

paresis all of the patients showed a marked change in the *activity pattern* on maximum effort, suggesting a considerable loss of muscle fibres in that the action potentials from active fibres near the electrode are led off without appreciable interference by the action potentials from more distant fibres. In normals a corresponding innervation effort is accompanied by interfering activity.

It was also characteristic that an attack of paresis was accompanied either by the occurrence of *spontaneous activity* or by an increase in any such existent activity. This spontaneous activity consisted of the irregular occurrrence of action potentials of short duration (2–4 milliseconds). Mechanical irritability was always increased during the paresis.

Mean duration of the action potentials was always significantly lower during paresis than otherwise. In 3 of the 4 cases it was significantly below normal. The standard error of the mean duration for the number of potentials recorded was $\pm$ 0.4 millisecond. For a difference to be regarded as significant (99 per cent) a deviation of 1.65 millisecond is therefore necessary. There was no increased frequency of polyphasic potentials (*i. e.*, potentials with more than 4 phases). The *velocity of propagation* of the impulses over the muscle was about 4 m/sec. both before and during the paresis, *i. e.*, about the same rate as in normal muscles.

Before the paresis the findings were less uniform. The activity pattern on maximum, voluntary innervation did not differ from that found for normal muscles. In one of three cases there was ample spontaneous activity and in another some spontaneous activity in the form of brief potential discharges. The mean duration lay within the normal range of variation in one, it was significantly increased in one and decreased in another. There was no increased incidence of polyphasic potentials.

Effect of acetylcholine injected intra-arterially.[*] – Five of the patients (Nos. 6, 13, 14, 16 and Vånga X: 15) received acetylcholine intra-arterially by the technique described by BUCHTHAL & ENGBÆK (1948). The intra-arterial injection of acetylcholine in

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[*] Acetylcholine tests were carried out by Dr. Lise Engbæk (Institute of Neurophysiology, Copenhagen).

Table 14. *Electromyographic findings. M. biceps brachii. 36—37 C*

Patient's No.		Age in years at examination	Degree of activity [1]	Attack provoked by [2]	EMG No.	Spontaneous activity	Electromyographic pattern during maximal effort [3]	Mean action potential duration in normal muscles in msec	Variation in normal material of same age	Mean action potential duration in patient's muscles in msec	No. of potentials	Duration in per cent: of mean duration without attack	Duration in per cent: of mean duration in normal muscles
8	no attack	11	4		146	+	I—T [4]	7.8	6.6—9.0	10.3	41	100	132
	attack		2	II		0	S			7.0	45	68	90
12	no attack	61	5		141	+	I	12.2	10.6—13.8	8.7	33	100	71
	attack		2—5	I		+ +	T			7.3	47	84	60
13	abatement of attack	21	3		145b	(+)	S—T	8.7	7.2—10.2	8.6	33	100	99
	attack		1—2	I		+ +	S			6.7	32	78	77
16	no attack	38	5		147	0	I	10.8	9.3—12.4	9.5	46	100	88
	attack		1—2	II		+	S			6.9	66	73	64

[1] 1 = trace of contraction
2 = active movement possible only with gravity eliminated
3 = active movement possible against gravity
4 = active movement possible against gravity and resistance
5 = normal power

[2] I = rest after physical exertion
II = oral administration of 2.5—5 g potassium chloride

[3] I = interference, *i.e.* simultaneous recruitment of many fibres
T = transitory state, a pattern of interfering activity, but so reduced that the discharges from the fibre nearest to the electrode dominate the curve
S = single oscillations, *i.e.* individual action potentials can be led off without interference from the response of adjacent units

[4] possibly not maximal effort

suitable quantities produces a motor reaction consisting of an observable movement, as a rule of flexion of one finger or more. It is thus not a question of maximum contraction of the muscles studied. The sensitivity was determined by examining what quantities of acetylcholine were necessary to produce a motor response. In addition, a vasomotor reaction occurs in the form of vasodilatation with lower threshold than the motor response and therefore provides a good possibility of checking that the acetylcholine was injected intra-arterially.

All of the patients with adynamia episodica hereditaria that were examined showed an increased sensitivity to acetylcholine both during attacks and free intervals. The results are given in Table 15. With this technique 100—300 micrograms is normally sufficient to produce a motor response in males and 150—250 micrograms in females (Engbæk 1951). Patients Nos. 6, 13, 14, 16 and Vånga X: 15 were tested during a free interval, Nos. 13 and 16 also during an attack.

Table 15. *Motor response to intra-arterial injection of acetylcholine*

Acetylcholine microgram	Patient No. 6 (female)	Patient No. 13 (male)		Patient No. 14 (male)	Patient No. 16 (female)		Patient No. Vånga X: 15 (female)
	between attacks	during attack	between attacks	between attacks	during attack	between attacks	between attacks
20			—				
30				(+)?			
50		+	+	+	—	(+)	—
100	+				+	+	+
150						+	

Spontaneous activity in the form of brief potential discharges and an activity pattern on maximum contraction in the form of single oscillations are seen mainly in patients with paresis of neurogenic origin (Buchthal & Clemmesen 1941, Buchthal & Pinelli 1952 and 1953 b). Decreased duration of the potentials occurs in myopathies (Kugelberg 1947; dystrophia musculorum progressiva: Kugelberg 1949; Buchthal & Pinelli 1952; Pinelli & Buchthal 1953; polymyositis: Buchthal & Pinelli 1953 a).

It is difficult on this basis to find an explanation for all the

changes observed. However, it is hardly justified to interpret the electromyographic findings in an acute transient attack of paresis in the same way as those occurring in chronic neurogenic or myogenic paresis.

Single oscillations on maximum attempt at innervation and the occurrence of action potentials of short duration can be explained on the basis of a blockage of many muscle fibres whereby some parts of the muscles are thrown entirely out of function, while in other parts of the muscles the potentials led off are derived from a smaller number of fibres than normally.

The spontaneous activity observed and its increase during paresis suggests a hypersensitivity of the fibres not completely blocked.

The conduction of impulses over the muscles is not changed, as is apparent from the normal velocity of propagation.

A normal electromyogram of a patient who probably had adynamia episodica hereditaria was described by Ecker & Carson (1953). However, it is not clear from their paper whether the electromyogram was traced during an attack or a free interval, probably during a free interval.

It might be of interest in this connection to mention two investigations of the electromyographic findings in periodic paralysis. Eichler, Jantz & Jung (1940) found the summation potential on maximum electric stimulation of the nerve to be prolonged and concluded that the rate of propagation of the impulse over the muscles was decreased.

Petersén & Widén (1955) described the electromyographic changes in a case of sporadic periodic paralysis. They found the duration of the potential to be shorter during the paralysis. In contrast to what is seen in adynamia episodica hereditaria, no spontaneous activity in the form of transient potentials was found in periodic paralysis, and on active contraction during paresis the number of potentials recorded was large in spite of the muscular weakness. The frequency of polyphasic potentials was also increased.

Increased sensitivity to acetylcholine has been found to occur in congenital myotonia, amyotrophic lateral sclerosis and traumatic

lesions of the peripheral nerves, in the two last-mentioned conditions due to prolonged denervation of the affected muscles. Reduced sensitivity occurs in progressive muscular dystrophy and myasthenia gravis (ENGBÆK 1951).

The increased sensitivity to acetylcholine might be ascribed to a specific change in the reaction of the end plate membrane as, for example, in chronically denervated muscles, or to an increased sensitivity of all of the muscle fibres, which also produces an increased sensitivity to the electrical change in the end plate region due to acetylcholine. The present investigation permitted no conclusion as to which of these two possibilities should be regarded as the more probable.

Unsuccessful Attempt at Provocation

Rest after physical exertion failed to precipitate an attack on 3 occasions (twice in one patient and once in another) as did the administration of potassium in 1. The potassium determinations made during 3 of the attempts are given in Table 16, in the fourth attempt no determinations were made. In this table the series are accounted for completely, because no value can be selected according to the clinical condition of the patient. After the administration of potassium the serum potassium increased slightly and exceeded the maximum value found during an attack in the same patient. During rest after exertion without an attack the serum potassium varied without any definite tendency, and in both patients the initial value was the highest.

Table 16. *Serum potassium during unsuccessful provocation (mEq/l)*

Patient's No.	Serum potassium										Remarks
	Initial value	Time in hours from end of exertion or potassium administration									
		0	½	1	1½	2	2½	3	3½	4	
Provocation I											
16d	4.7	4.0	4.0	4.4	4.4						Walking 1 hr 30 min
17d	4.9	4.6	4.7	4.6	4.3	4.2	4.0	4.5			Walking 1 hr 30 min
Provocation II											
7d	4.6	—	4.9	4.8	4.8	4.9	4.8	4.8	4.9	4.8	4 g KCl

Diagnosis and differential diagnosis

Diagnosis

In adynamia episodica hereditaria the typical history, described in detail on page 48, is often sufficient to establish the diagnosis. In the present investigation it was also shown that the disease is inherited as an autosomal, monohybrid, dominant with complete or almost complete penetrance (see page 36). Therefore, knowledge of the fact that the disease almost always occurs in one of the parents and his or her family can in future be useful in the establishment of the diagnosis.

The diagnosis can only be confirmed by precipitation of an attack, since examination between attacks will, as a rule, show nothing of interest. An attack can be precipitated by rest after physical exertion or by oral administration of potassium. This should preferably be done first thing in the morning before breakfast. The former method, which coincides with the way in which the attack is precipitated under ordinary conditions, involves no risks. The latter is, however, less time-consuming and the severity of the attack can be varied by adjustment of the dose of potassium. The administration of potassium chloride can involve certain risks owing to individual differences in sensitivity to the substance. Therefore, if this procedure is adopted, calcium should be at hand for intravenous injection. The initial dose should not exceed 4 g of potassium chloride for adults, 3 g for older children, and 2 g for small children. If this dose fails to elicit an attack, a second dose, but not on the same day, 1 g larger than the first, is administered.

In the present investigation administration of 2–3 g potassium chloride always precipitated an attack in the children, and 4 g in all of the adults except one. This adult patient could not spare a second day for a subsequent test with a larger dose. In another patient who received 4 g potassium chloride the attack was mild, and after 5 g of medium severity. In none of the 26 unaffected persons did oral administration of 3–5 g of potassium chloride produce any demonstrable reaction (See pages 54 and 100). In

investigations in the U. S. A., where 12.5–15 g potassium chloride was given to healthy test subjects, slight muscle weakness was observed only in exceptional cases and never were the muscular symptoms severe (KEITH et al. 1942, DODGE et al. 1953). The reaction of a patient with adynamia episodica hereditaria to a small dose of potassium is thus of diagnostic value. Whether the diagnosis can be excluded in those cases in which the administration of 5 g of potassium chloride does not produce paresis, can at present not be decided. It is possible that an investigation on a larger series might reveal cases in which doses larger than 5 g of potassium chloride are necessary for provoking attacks.

Patients with impaired renal or adrenocortical function can react with grave symptoms to a moderate dose of potassium (SMILLIE 1915, FINCH & MARCHAND 1943, MARKS & FEIT 1953). Since the administration of potassium in such cases might involve undue risks and since the reaction is difficult to evaluate, it should not be employed in the presence of signs or symptoms of renal or adrenocortical injury.

In view of the toxic effect of potassium on the heart, the test should be performed only with great caution on patients with heart disease.

Clinical examination during an attack is not always sufficient to establish the diagnosis with certainty, because reflex disturbances and a positive Chvostek's sign are by no means regular. Characteristic of an attack of adynamia episodica hereditaria are an increase in the serum potassium and electrocardiographic changes; high and pointed T-waves and tachycardia. The changes disappear before or together with the abatement of the paresis. Since the serum potassium need not increase beyond the upper normal limit and since electrocardiographic changes of this type can hardly be evaluated on the basis of a single electrocardiogram, blood samples and electrocardiograms should be taken on at least 3 different occasions: before the attack, when the attack is at its maximum and during abatement of or after the attack. These disturbances in serum potassium and electrocardiographic changes are, however, occasionally missing in prolonged and less well defined attacks.

Differential diagnosis

The historical background in *familial periodic paralysis* varies so widely that in a given case this disease cannot always be distinguished from adynamia episodica hereditaria on the basis of historical data only. In a large series, however, differences between the characteristic features of the two diseases can be recognised.

In adynamia episodica hereditaria inheritance is due to a single, autosomal dominant gene with complete or almost complete penetrance, while in families with periodic paralysis it is claimed as being due to a recessive (TALBOTT 1941), as due to a dominant with complete (GAUPP JR. 1940, TALBOTT 1941, MEYER 1952) or incomplete penetrance (MYERS 1949, SAGILD & HELWEG-LARSEN 1955) or as due to a sex-linked recessive (KHAN 1935).

TALBOTT's opinion that the mode of inheritance of familial periodic paralysis can be recessive is based on the fact that the disease can skip one or more generations and then appear again. Such skipping does not, however, exclude the possibility of dominant inheritance, and TALBOTT's view of a recessive inheritance of periodic paralysis cannot be regarded as established. It should also be pointed out that his material consists of cases described in the literature over a period of more than 50 years. Familial investigations have been carried out to a varying extent by different authors, so that it is possible some of the members of the families with a mild form of the disease were never discovered and reported.

MYERS' (1949) material consisted of 25 cases in 6 generations. Only one generation, and then only one member, was skipped, namely a healthy female who had transmitted the disease to 5 of her 7 children.

SAGILD & HELWEG-LARSEN's (1955) series of 50 patients has not yet been presented in its entirety. One pedigree published includes 1 affected female and 11 affected males. The disease was transmitted 3 times through healthy women and 3 times through healthy men.

KHAN (1935) based his opinion of a sex-linked recessive in-

heritance on the observation, in an Indian family, of 8 affected males and no affected females. The trait was transmitted 3 times – from maternal grand-father to grandson – through unaffected women. However, the fact that one generation was skipped does not exclude dominant inheritance. In addition, the possibility of the disease being concealed among the females must be taken into account. It should be stressed that KHAN is the only author who expressed the view that familial periodic paralysis is inherited as a sex-linked recessive.

Adynamia episodica hereditaria is just as common in both sexes; familial periodic paralysis is more common among males (TALBOTT 1941, SAGILD & HELWEG-LARSEN 1955), according to SAGILD & HELWEG-LARSEN (1955) because of decreased penetrance among women. Since the material of the last-mentioned authors has not been published in its entirety, it is not yet possible to judge the validity of their opinion.

In adynamia episodica hereditaria the onset usually (90 per cent) occurs within the first decade of life; in familial periodic paralysis, usually in the second decade (DALINGHAUS 1941, TALBOTT 1941).

In adynamia episodica hereditaria the attacks usually occur about once a week and last for about an hour, in periodic familial paralysis the attacks are less frequent – about once a month – but of longer duration – usually 6 to 48 hours (DALINGHAUS 1941, TALBOTT 1941, MYERS 1949, SAGILD & HELWEG-LARSEN 1955).

In adynamia episodica hereditaria the attacks are more common during the day than at night, while in familial periodic paralysis nocturnal attacks are the rule (CERNY & KATZENSTEIN-SUTRO 1952).

During an attack of adynamia episodica hereditaria the paresis is usually only slight or moderate without complete loss of mobility: the reflexes can be weak or absent, but are not regularly so, and the electric excitability is preserved. In familial periodic paralysis the patient is, as a rule, completely helpless, the tendon reflexes are absent and the musculature is electrically inexcitable on direct or indirect stimulation (DALINGHAUS 1941, TALBOTT 1941).

As far as is known, no patient has ever died of adynamia episodica hereditaria. Periodic paralysis can, however, be fatal. Thus, TALBOTT (1941), who analysed the literature cases until that time, found that of the 400 cases on record, death had followed an attack in more than 35.

The most important examination in the differentiation between the two types of paralysis is a study of the electrolytes during an attack. In adynamia episodica hereditaria there is usually an increased serum potassium accompanied by hyperpotassemic electrocardiographic changes. In familial periodic paralysis there is, as a rule, decreased serum potassium accompanied by hypopotassemic electrocardiographic changes (BIEMOND & DANIELS 1934, TALBOTT 1941, JANTZ 1947). In adynamia episodica hereditaria attacks can be provoked by the administration of potassium, while glucose has a good prophylactic effect. In familial periodic paralysis attacks can be provoked by the administration of glucose, while potassium has a good prophylactic and/or therapeutic effect (AITKEN et al. 1937, JANTZ 1947, MCQUARRIE & ZIEGLER 1952).

Typical of *myasthenia gravis* is increased fatigability of the striated muscles, *i. e.*, the patient becoming weaker and weaker on exertion and recovering during rest, and involvement first – and most severely – of the musculature innervated by the cranial nerves (ANTONI 1947). Thus in these respects the clinical picture differs distinctly from that of adynamia episodica hereditaria. The normal reaction to d-tubocurarine in adynamia episodica hereditaria consistutes another difference between the two diseases, because patients with myasthenia gravis are about 10 times more sensitive to d-tubocurarine (BENNETT & CASH 1943).

The typical inability to relax after a muscle contraction in *myotonia congenita* is not seen during or between attacks of adynamia episodica hereditaria.

In one of the patients (No. 10) examined in hospital there was weakness of the legs and arms also between the attacks as well as muscle atrophy. Microscopic examination of a biopsy specimen of a muscle showed changes of the type described in cases of *dystrophia myotonica* (WOHLFART 1951). This patient had not cataract, and showed no evidence of endocrine disturbances con-

mon in dystrophia myotonica, which argues against the coincidence of the 2 diseases. Neither was cataract seen in any of the other 15 patients examined ophthalmologically, and muscle atrophy only in the aforementioned patient No. 10. It should also be stressed that the muscle biopsy specimens from 2 other patients examined in hospital (Nos. 13 and 16) were of normal appearance. These 2 patients were, however, younger and had not had the disease for such a long time.

In *myoglobinuria*, described by Hed (1955) also in its familial form, attacks of muscle weakness can occur on physical exertion or insufficient carbohydrate intake. Unlike adynamia episodica hereditaria, in myoglobinuria the attacks occur during exertion and not after a period of rest. In addition, the picture is dominated by muscle pain and tenderness and not by paresis. Finally, during the attacks the urine excreted is dark and gives a positive benzidine reaction.

Under the title of *Myopathy due to a defect in muscle glycogen breakdown* McArdle (1951) described another type of transient muscle weakness. Typical of that condition was that – in contrast to what is seen in adynamia episodica hereditaria – the weakness occurs during physical exertion, is accompanied by cramp-like pain and abates during rest.

Two types of paralysis can occur in attacks of *hypoglycemia.* In prolonged hypoglycemic coma brain injury can develop and cause hemiplegia, which abates with the other symptoms of brain injury (Silfverskiöld 1946). After hypoglycemic coma paresis of the extremities and muscle atrophy can occasionally, though rarely, persist for weeks to months: according to Silfverskiöld (1946), because of injury to the peripheral nerves; according to Tom & Richardson (1951), because of degeneration of the anterior horn cells of the spinal cord. Both types of paresis thus differ distinctly from that seen in adynamia episodica hereditaria, in which repeated blood sugar determinations between and during attacks were found to be normal.

In attacks of *porphyria acuta* the neurologic symptoms include paresis of varying localisation and spread, but these attacks are dominated, at least in the initial stage, by general discomfort and

severe abdominal symptoms: pain, vomiting and constipation (WALDENSTRÖM 1939).

Thyroidal hypofunction or hyperfunction is occasionally accompanied by neuromuscular disorders such as chronic myopathy, and in hyperfunction periodic paralysis may occur, though rarely (MILLIKAN & HAINES 1953). None of the patients with adynamia episodica hereditaria showed clinical signs of thyroidal disorders, and of 13 in whom the B. M. R. was studied, it was somewhat low in 1 and normal in the remainder.

Hyperpotassemia, occasionally, though rarely, accompanied by flaccid paresis which disappears as soon as the serum potassium returns to normal, can occur in severe *diseases of the adrenocortex or of the kidneys* with decreased urinary excretion of potassium (BULL et al. 1953, RICHARDSON & SIBLEY 1953). In such states the patient is in a poor general condition and shows distinct signs of the fundamental disease. In adynamia episodica hereditaria, however, the patient's general condition is good. These patients lead an ordinary life, working capacity is at most slightly impaired and even during an attack their general condition is at most moderately affected. The excretion of potassium is normal and does not decrease during an attack.

Course and prognosis

Course. — The typical course of the disease is apparent from what was said about the variation with age on page 53. In brief, during childhood the attacks are short and frequent; during puberty and the next decade the frequency remains more or less the same, but the attacks are then more severe and longer. After 30 years of age, the attacks become less frequent and less severe in about half of the patients, but they do not change significantly in duration. However, the following exceptions to this course were noted. In only 1 patient, aged 60, did the disease progress after 30 years of age, and he gradually developed permanent paresis and muscle atrophy. Another patient, also aged 60, had slight, permanent weakness of the arms, but without atrophy. The attacks were, however, less frequent and less severe than a few decades before.

One woman, in whom the disease initially appeared at the age of 31, had attacks only during pregnancy. In the mildest cases, with only one or a few attacks per year, any variation in the severity was too slight to be demonstrable.

Prognosis. — As far as the individual attack is concerned, the prognosis is good. As yet no attack has been known to be fatal and the paralysis always disappeared spontaneously. As to the prognosis of the disease as such, an improvement occurs in about half of the patients after the age of 30, especially among the moderate cases. Permanent muscle weakness was observed only in the 2 aforementioned patients. The disease never led to complete loss of working capacity, not even in the 2 patients with permanent paresis.

Risk of affection. — The risk of children from affected × unaffected matings inheriting adynamia episodica hereditaria is 50 per cent. There is hardly any risk of children of unaffected sibs inheriting the condition. However, should a child of an apparently unaffected relative show symptoms of the disease, it may be concluded that the parent is a genetic carrier of the disease.

Therapy

As yet no effective treatment of adynamia episodica hereditaria *per se* is available.

In the treatment of the individual attacks intravenous injection of calcium proved of some value. If the injection was given when the attack was at its maximum, as a rule, it rapidly controlled the paralysis. Oral administration of calcium citrate (1 g 3 times daily) over a longer period had no beneficial effect.

As a rule, oral pre-medication with glucose prevented the precipitation of an attack by an otherwise provocative dose of potassium. Once an attack had started, however, glucose by mouth or intravenously by itself or in combination with insulin had no definite beneficial effect.

The patients themselves stated that they were troubled less by their disease if they followed a regimen consisting of moderate

physical exertion, sufficient sleep, and regular meals at fairly short intervals. If the patient eats or does his utmost to exercise the limbs during an attack, he can "eat off" or "work off" the paralysis quicker than if he simply lies still. Passive change in the position of the body during the severest stage of the attack will give some relief.

Results of corresponding studies in controls

The age and sex distribution of the 18 controls is given in Table 3. One of the controls (S) was a healthy member of the Vånga family.

The serum potassium, the excretion of potassium in the urine and in some cases the electrocardiograms were studied during 2–3 hours' rest after physical exertion or during 2–4 hours after oral administration of 3–5 g of potassium chloride. The tests were always carried out before breakfast. In some of the subjects the administration of potassium was followed by transient nausea and mild epigastric pain. No other side effects were noted. Examination of the gross functional strength and reflexes revealed no signs of a pathologic condition. The results of blood studies and electrocardiography are given in Table 17; of urinalysis, in Table 18.

Serum potassium

Rest after physical exertion. — Some variation was found in the serum potassium values recorded during rest after exertion. The variation was small and irregular and the serum potassium lay within normal limits. The highest value noted was 5.0 mEq/l. Similar observations were made in patients with adynamia episodica hereditaria during rest after exertion, provided no attack was precipitated (see Table 16). In the event of an attack the behaviour of the serum potassium is quite different (Table 11). The serum potassium curve for subjects A and F (this last series was, however, not complete), slightly resembled that seen in attacks of adynamia episodica hereditaria. In the other 11 it was strikingly different.

Table 17. *Serum potassium (mEq/l) and electrocardiogram in controls*

Control	Serum potassium									ECG	Remarks
	Initial value	Time in hours from end of exertion or potassium administration								no changes − changes +	
		0	½	1	1½	2	2½	3	3½		
During rest after exertion											
A	4.0	4.4	4.1	4.2	4.6	4.4	4.1	4.1			Playing out-of-doors 2 hr
B			4.7	4.4	4.8	4.6	4.7	4.8			Playing out-of-doors 2 hr
F	4.5			4.8		4.8	4.6	4.2			Playing out-of-doors 1 hr 15 min
I	4.3	4.2	4.5	4.2	4.2	4.1	4.1				Cycling 1 hr 30 min
K	4.4	4.4	3.8	3.8	4.4	3.8					Cycling 1 hr 30 min
L	4.2	4.2	4.1	4.2	4.2	4.7	4.5				Cycling 1 hr 30 min
M	4.2	4.2	4.3	4.1	4.3	4.3	4.7				Cycling 1 hr 30 min
N	4.0	4.0	4.0	3.9	4.4	4.0	4.2				Cycling 1 hr 30 min
O	4.4	4.3	4.4	4.5	4.5	4.7	4.4				Walking 1 hr 30 min
P	4.4	4.2	4.4	4.4	4.4	4.6	4.4				Cycling 1 hr 30 min
Q	4.6	4.4	4.8	4.6	4.8	4.8	5.0				Cycling 1 hr 30 min
R	4.4	4.4	4.4	4.4	4.3	4.2	4.2				Cycling 1 hr 30 min
S	3.8	3.8	4.2	4.0	4.0	4.2	4.0			—	Cycling 1 hr 30 min
After potassium administration											
B	4.5		5.1	5.0	5.3	4.8	4.8	5.1		+	3 g KCl
C	4.4			5.8		5.6				+	4 g KCl
D	5.7			5.9		5.5					4 g KCl
E	4.6		5.0	4.9	4.9	5.3	5.3			+	3 g KCl
F	4.8		4.8	4.7	5.9	5.6	5.5	5.0		+	5 g KCl
G	5.2			6.0		5.8				+	4 g KCl
H	4.7			6.0		5.0					4 g KCl
I	4.2		4.6		4.9				4.8		4 g KCl
K	4.6		4.6		5.2		4.0		4.5		4 g KCl
L	4.4		4.6		5.0		4.8		4.7		4 g KCl
M	4.4		4.9		4.8		4.6		4.3		4 g KCl
N	4.2		4.2		5.2		4.6		4.4		4 g KCl
O	4.0		4.3		4.3		4.5		4.0		4 g KCl
P	3.8		4.2		4.7		4.4		4.2		4 g KCl
Q	4.8		4.8		4.8		5.0				4 g KCl
R	4.4		4.6		5.1		4.7		4.7		4 g KCl
S	5.1		4.5	4.9	4.8	4.5	4.5	4.2			4 g KCl

The increase in serum potassium in association with an attack of adynamia episodica hereditaria and the decrease after the attack to a level below the initial was thus a mode of reaction specific of the attacks of this disease and did not occur in healthy persons during rest after physical exertion.

After oral administration of potassium. — The administration of potassium chloride was followed by an increase in serum potassium in all of the subjects except one. The increase reached a maximum thirty minutes to two hours and a half after the administration of potassium. The highest value recorded was 6.0 mEq/l and the increase was on the average 0.75 mEq/l (the series with decreasing serum potassium was thus not included). A corresponding, but usually somewhat larger, increase was noted for the patients, in whom in 15 of 21 attacks the serum potassium at the maximum of the attack was more than 6.0 mEq/l. In addition, the increase in serum potassium in the patients was accompanied by an attack of paralysis, an effect not seen in any of the controls.

The observations made in the controls were in line with the report of Keith & Osterberg (1946), on administration of a similar dose of potassium.

The patients thus differed from the controls in that they reacted with a larger increase in serum potassium and, secondly, that paresis developed at some level, at which no symptoms appeared in controls.

Urinary excretion of potassium

The excretion of potassium in the urine varied widely among the controls as well as among the patients. During rest after exertion the excretion in 10 controls lay between 0.2 and 3.5 with an average of 2.0 mEq/hr. In the patients, on the other hand, the excretion in attack I was on the average 6.0 mEq/hr and only in 2 attacks was it less than 3.5 mEq/hr. After the administration of potassium to the controls the variation in the excretion was wider, and in 15 tests it lay between 0.7 and 31 mEq/hr with a mean of 8.8 (if the extreme value of 31 mEq/hr is not included, the figures will be 0.7 to 12.2 with a mean of 7.2). In the patients the excretion during attack II was on the average 7.5 mEq/hr. Thus, neither during rest after exertion nor after the administration of potassium did the excretion of potassium tend to be lower among the patients than among the controls.

The increase in serum potassium found in the patients can thus not be ascribed to decreased excretion of potassium in the urine.

Table 18. *Urinary potassium (mEq/hr) in controls*

Control	During rest after exertion	After administration of potassium	Control	During rest after exertion	After administration of potassium
C		7.7	M	0.5	0.7
D		31	N	2.4	2.8
E		4.8	O	2.5	5.0
G		9.7	P	0.2	12.2
H		10.8	Q	1.9	12.2
I	1.2	5.0	R	2.8	8.8
K	3.5	5.8	S	1.7	9.7
L	3.1	5.9			

Electrocardiographic studies

The electrocardiograms of the healthy subjects showed the same type of changes in the shape and height of the T-waves as for the patients.

Electrocardiographic changes in the presence of hyperpotassemia were first described by WINKLER, HOFF & SMITH (1938). They produced hyperpotassemia in dogs by injecting potassium chloride solution intravenously, during which they recorded electrocardiograms. The initial changes were observed with a serum potassium level of 5.0–7.8 mEq/l and consisted of an increase in the amplitude of the T-waves. When the serum potassium increased still more, the S–T interval was depressed, and the P-waves disappeared.

THOMSON (1939 a, b) made similar observations in human beings. He examined patients with uremia and Addison's disease and healthy persons after the administration of potassium, and found the commonest accompaniment of increasing serum potassium to be an increase in the height of the T-waves. In a few cases intraventricular block occurred. The serum potassium level at which the electrocardiographic changes appeared varied from one patient to another.

High, pointed T-waves and in more serious cases prolonged conduction time, loss of P-waves and intraventricular block were described in hyperpotassemia of different origins by FINCH & MARCHAND (1943), KEITH & OSTERBERG (1946), STEWART, SHEPARD

& Horger (1948), Keith & Burchell (1949), Ljung (1949), Nicholson & Spaeth (1949), Merrill et al. (1950), Bull et al. (1953), Meroney & Herndon (1954). Tarail (1948) pointed out that the electrocardiographic changes appear at a serum potassium level, varying from one patient to another, and that in a given patient they occur simultaneously with variations in the serum potassium. Westlake & Chiu (1954) claimed that hyperpotassemia cannot be diagnosed with any degree of certainty on the basis of a single electrocardiogram unless the serum potassium level exceeds 7.3 mEq/l.

The electrocardiographic changes observed during attacks of adynamia episodica hereditaria cannot be regarded as a specific sign, since they occur in association with increasing serum potassium independently of the cause of the increase.

SUMMARY

A disease characterised mainly by attacks of spontaneously abating paralysis, particularly of the musculature of the extremities and the trunk, is described under the name of *adynamia episodica hereditaria.*

One hundred and thirty-eight patients with the disease are known. Sixty-eight were examined by the writer. Of these, 17 were examined in hospital, where they were observed both between and during attacks, and where various methods of preventing, precipitating and controlling the attacks were tried.

Mode of inheritance

The disease was found to be hereditary.

The inheritance is due to a single, autosomal, dominant gene with complete or almost complete penetrance.

Anamnestic

In more than 90 per cent of the patients the onset occurred below the age of 10 years, and only in 1 above the age of 30 (108 patients interviewed).

The attacks occurred during rest after exertion (in all patients).

In about three fourths of the patients the attacks occurred at least once a week, and in roughly the same number the attacks lasted at most 1 hour (94 patients interviewed).

The extent and severity of the paresis varied from slight weakness of a single extremity to severe states in which the patient, when lying, was unable to turn over or sit up without help. The respiratory muscles were seldom involved, and when, only slightly (7 out of 91 patients). Mild symptoms from the musculature innervated by the cranial nerves had occurred at least once in about half of the patients (92 patients interviewed).

Gentle exercise (walking or moving about) at the appearance of the first symptoms of an attack or as soon as possible during an attack always gave relief.

The intake of food, especially of bread, at the onset or during an attack warded off, or shortened, the duration of the symptoms (56 out of 84 patients).

The disease was found to be equally common among males as among females (73 : 65), but the course was on the whole somewhat more severe in the males.

During childhood the attacks were short and frequent, at puberty they became longer and more severe though not more frequent. The disease was usually most troublesome between puberty and about 30 years of age, after which improvement occurred in about half of the patients (40 patients interviewed). Only 1 patient reported progression of the disease after 30 years of age. In a few of the patients the attacks ceased to occur after the age of 50–60 (3 out of 11 patients).

The disease was more troublesome in cold, damp weather (64 out of 94 patients).

The attacks were always more common during the day-time than at night.

Working capacity was never more than slightly reduced. According to information from relatives, no patient has died from an attack of adynamia episodica hereditaria.

Findings

Only exceptionally did clinical examination, blood studies or electrocardiography between attacks reveal anything of interest.

During attacks paralysis of varying extent and severity was observed, and in somewhat more than half of the attacks tendon reflexes were weak or absent (36 out of 61 attacks). In somewhat less than half (25 out of 61 attacks) Chvostek's sign was positive.

As a rule, the attacks were accompanied by an increase in the serum potassium (45 out of 49 attacks) without any decrease in the excretion of potassium in the urine and by such electrocardiographic changes as are seen in the presence of increasing serum

potassium independently of the cause of the increase (38 out of 47 attacks).

Attacks could nearly always be precipitated by oral administration of a dose of potassium not large enough to produce symptoms in healthy persons (25 attacks precipitated in 16 patients, only 1 unsuccessful attempt).

Calcium administered intravenously at the culmination of an attack usually soon controlled the symptoms (10 out of 11 attacks).

Glucose with or without insulin administered before, or simultaneously with, an otherwise provocative dose of potassium had a prophylactic effect, the attack then being prevented (3 tests) or less severe than otherwise (2 tests).

Electromyographic changes occurred during an attack in the 4 patients studied: the innervation pattern changed suggesting loss of muscle fibres; in 3 spontaneous activity occurred or, if present, increased. In all 4 the mean action potential duration was significantly lower than before the attack.

The threshold of motor response to the intra-arterial injection of acetylcholine was low both between and during attacks (5 patients studied).

The attacks abated spontaneously. Only in 2 patients, now aged 60, was permanent, slight muscle weakness observed between attacks, and only in 1 was there any muscle atrophy, and then only slight. Histologic examination of a biopsy specimen of the muscle of the latter patient showed changes of the type seen in dystrophia myotonica. Histologic examination of muscle tissue from 2 younger patients without atrophy or permanent muscle weakness showed no evidence of a pathologic condition.

As yet, no effective treatment of the disease *per se* is available.

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SURVEY OF GENETIC AND CLINICAL DATA

Table A. *Survey of genetic data.*

Pedigree number: V = Vånga, M = Matteröd. Compare pedigree charts (page 112 and 113).

State of health: affected members are given in italics. State of health unknown is indicated by x (for members) or y (for parents not belonging to the family).

A dot (.) indicates that the individual, the birth year or the age is unknown.

Every mating is accounted for separately. Double commas indicate that the same individual is included in more than one mating. An exception to this rule is the family branch Vånga VI:1, in which a group of half-sibs, about whose fathers nothing is known, are taken together.

Indices after x: 1 = affected descendants known. 2 = descendants known, none affected. 3 = no children. 4 = attempts to trace descendants unsuccessful (information about descendants is given only for adult members).

Pedigree No. of the individual belonging to the family		Year of birth		State of health, sex of child, age at, and manner of, disappearance from observation		
Grand-parent	Parent	Father	Mother	Father	Mother	Children
—	V II:1 or 2	1699	1706	D85x	D63x	MU.x FD9x MU.x MD72x^1 MD71x^2 FD77x^1 MD41x^4 MD51x^1
V II:1 or 2	V III:4	1735	1740	D72x	D70y	MD77x^1FD74x^1 MD1x MD0x FD0x FD53x^2 FD10x
	V III:6	1734	1739	D61y	D77x	FD72x^1 MD0x FD70x^2 MD79x^2 FU25x^4
V III:4	V IV:1	1765	1776	D77x	D39y	FD76x^4 FD40x^1 FD77x^2 FD20x^3 MD20x^3
	V IV:2	1750	1767	D65y	D74x	MD38x^4 FD53x^1 FD80x^1
V III:6	V IV:8	1759	1767	D59y	D72x	MD0x FD69x^2 MD57x^1 MD35x^2 FD0x
V IV:1	V V:2	1801	1805	U71y	D40x	FD69x^1 FU36x^3 MD2x MD82x^3 MU26x^3 MU23x^3 FU46x^3
V IV:2	V V:7	1792	1801	D77y	D53x	*MD63* FD82x^4
	V V:8	1802	1806	D79y	D80x	*FD76* MD95 FU29x^3 *FD72* FD76x^3 FU11x FD91 *FU54*
V IV:8	V V:11	1797	1799	D57x	D80y	MD0x FD50x^1 FD0x FD0x *FU25* FU33x^3 MD0x MU20x^3
V V:2	V VI:1	.	1829	.	D69x	FD.x^3 FD58x^1 *FD67* *MD43* FD2x FU18x^3 MD73 Presumably half-sibs, nothing known about the fathers
V V:7	V VI:8	1822	1824	*D63*	D76	*FD86* FD66 FD74 *MD80* *MD68* FD84 FD78 *FD69*
V V:8	V VI:10	1827	1828	D75	*D76*	FD0 FD0 *FD60* *FD92* FD23

	V VI:13	1835	1836	D35	*D72*	MD83 *MD76* *FD38* FU21 ML84
	– „ –	1850	– „ –	D80	– „ –	*MD73* FD73 *FD64*
	V VI:17	1844	1848	U41	*U54*	FU20x^3 MU21x^3 FU28x^3 MU15x^3 MU22x^3 *MD71* FD3x FU16x^3
V V:11	V VI:19	.	1823	.	D50x	FD54
	– „ –	1820	– „ –	D86y	– „ –	MU29 FU41 *FD81* MD6
	V VI:22	1823	1828	U30	*U25*	*MD81* MU.*
V VI:1	V VII:2	.	1855	D.y	D58x	*FD74*
	– „ –	1823	– „ –	D76y	– „ –	MU22x^3
	V VII:3	1855	1859	U51	*D67*	FL64 *FL62* *MD34* *MD22* ML56 ML54
	V VII:4	1863	1866	*D43*	D.	MD21 *ML60* *FD27* MD0 *ML52* *ML49*
V VI:8	V VII:8	1850	1848	D75	*D86*	ML79 FD59x^1 *FL71* FL69 ML65 *ML60*
	V VII:11	1855	1860	*D80*	D43	FL70 *FL67* FL64 FL62 *MD27* ML58 MD0 *MD53* MD0
	V VII:12	1859	1865	*D68*	L89	ML62 FD1 *FL57*
V VI:10	V VII:18	1842	1854	D.	*D60*	MD0 MD67 FU21
	V VII:19	1848	1857	D58	*D92*	*MD49* *ML75* FD20 MD14 FL69 ML67 *FD42* FL62 FL60 *ML57* *FL55*
V VI:13	V VII:22	1862	1868	*D76*	L86	*FL64* FL62 MD6 *FL46*
	V VII:23	1858	1865	D81	*D38*	ML65 ML59 *ML57* ML55 *ML53* FL51
	V VII:26	1874	1884	*D73*	D61	*ML44* *ML38*
	V VII:28	1877	1879	D64	*D64*	ML51 FL47 ML43 ML38 FL38 *ML36*
V VI:17	V VII:34	1882	1873	*D71*	L81	*FL45* *ML43* *FL41*
V VI:19	V VII:40	1859	1857	D50	*D81*	*FL64* ML63 MD49 MD54 ML59
V VI:22	V VII:42	1851	1849	*D81*	D74	FL77 FD11 MD10 *ML61*
V VII:2	V VIII:1	.	1880	.	*D74*	ML53
	– „ –	.	– „ –	.	– „ –	FL50
	– „ –	1885	– „ –	L69	– „ –	ML48 ML46 ML45 MD16 FL43 FD5 ML39 *FL38* *FL34* ML30
V VII:3	V VIII:4	.	1892	U26	*L62*	*FD23*
	V VIII:5	1894	1903	*D34*	L51	FL31
V VII:4	V VIII:10	1894	1894	*L60*	L60	*ML30* *FL29*
	V VIII:11	.	1897	U.	*D27*	*ML32*

* Exact age unknown, certainly above 20, but probably below 30.

Pedigree No. of the individual belonging to the family		Year of birth		State of health, sex of child, age at, and manner of, disappearance from observation		
Grand-parent	Parent	Father	Mother	Father	Mother	Children
V VII:8	V VIII:16	1873	1878	D49	D59x	MD3 *MD48* FD37 *FL49* *FL47* ML42 FL40 *FL38* FL34
	V VIII:17	1878	1883	L76	*L71*	FL42 *ML41* FL38 *ML37* ML35 FL34 *FL32* FL30 ML28 *ML26*
	V VIII:20	1894	1899	*L60*	D53	*ML34* FD1
V VII:11	V VIII:22	1881	1887	D66	*L67*	*ML45* *ML43* FL42 ML40 *ML38* *ML34* *FL32*
	V VIII:28	1900	1902	*D53*	L52	FL27 *ML20* FL15
V VII:12	V VIII:32	1891	1897	D40	*L57*	ML23
V VII:19	V VIII:36	1878	1888	*D49*	L66	ML41 FL38 FL34 FL31
	V VIII:37	1879	1883	*L75*	D40	FL47 *FL45* FL42 FL40 ML38 *FL36*
	V VIII:42	.	1890	U.	*D42*	*FL40*
	V VIII:45	1897	1897	*L57*	D34	*FL32* *ML31* FL28 *ML26* *ML23*
	– ,, –	– ,, –	1913	– ,, –	L41	*FL20*
	– ,, –	– ,, –	1896	– ,, –	L58	*ML17* *ML14*
	V VIII:46	1896	1899	L58	*L55*	ML35 *FL32* FL28
V VII:22	V VIII:47	1885	1890	L69	*L64*	FL44 *ML42* *FL40* *ML34* *FL32* ML29 ML24
V VII:23	V VIII:53	1897	1901	*L57*	L53	ML24 *FL21* *ML19* ML16 ML15 FL7
	V VIII:55	1901	1914	*L53*	D22	*ML24* *ML21*
V VII:26	V VIII:57	1910	1913	*L44*	L41	FL13 *ML11* FL9 ML5
	V VIII:58	1916	1917	*L38*	L37	*FL11*
V VII:28	V VIII:64	1918	1922	*L36*	L32	FL10
V VII:34	V VIII:65	1907	1909	L47	*L45*	ML22 ML19 *FL16* ML14
	V VIII:67	1910	1913	L44	*L41*	*ML20* FL10 ML3
V VII:42	V VIII:76	1893	1898	*L61*	L56	*FL37*
V VIII:1	V IX:10	1917	1916	L37	*L38*	*ML16* *ML14* FL13 ML9 ML8 ML3
	V IX:11	1914	1920	L40	*L34*	*FL14* ML10
V VIII:4	V IX:13	.	1918	U.	*D23*	*ML18*
	– ,, –	1915	– ,, –	L39	– ,, –	*FL13*

V VIII:10	V IX:15	1924	1929	*L30*	L25	ML6
	V IX:16	1926	1925	L28	*L29*	*ML5* ML1
V VIII:16	V IX:19	1901	1903	*D48*	L51	ML25 *FL23* FL21 *FL12*
	V IX:21	1897	1905	L57	*L49*	ML22 ML19
	V IX:22	1906	1907	L48	*L47*	ML12 FL9 *ML6*
	V IX:25	1913	1916	L41	*L38*	ML18 ML7 FL3
V VIII:17	V IX:28	1913	1916	*L41*	L38	FL7 FL2
	V IX:33	1913	1922	L41	*L32*	*ML9 FL7*
V VIII:20	V IX:37	1920	1921	*L24*	L33	*ML10*
V VIII:22	V IX:39	1909	1917	*L45*	L37	*FL14 ML10* FL7
	V IX:40	1911	1922	*L43*	L32	FL11 ML9 ML5 FL0
	V IX:44	1920	1930	*L34*	L24	ML1
	V IX:45	1919	1922	L35	*L32*	ML9 ML6
V VIII:37	V IX:55	1914	1909	L40	*L45*	*ML18 FL14* ML9 ML5
	V IX:59	1910	1918	L44	*L36*	ML10 ML9 FL6 ML0
V VIII:42	V IX:60	1903	1914	L51	*L40*	FL16 FL13
V VIII:45	V IX:61	1921	1922	L33	*L32*	*FL12* FL10 FL9 *ML7* ML5 FL1
V VIII:46	V IX:70	.	1922	U.	*L32*	ML6
V VIII:47	V IX:73	1912	1917	*L42*	L37	FL15 *FL13 FL7* ML5
	V IX:74	1907	1914	L47	*L40*	*ML17 FL12*
	V IX:75	1920	1919	*L34*	L35	*FL13 FL9* ML3
	V IX:76	1920	1922	L34	*L32*	ML8 FL1
V VIII:76	V IX:100	1915	1917	L39	*L37*	ML11 *ML10 ML5* FL3
—	M I:1 or 2	1780	1790	D.	D.	FD60 FD44 FD56 MD27 *MD59* MD17
M I:1 or 2	M II:5	1826	1836	*D59*	D53	MD3 *MU50 MD80 MU25* ML84 *FD28*
M II:5	M III:2	1862	1862	*U50*	D49	*FD16 MD20 FD3* MD15 *MU14* MU12 FD2
	M III:3	1864	1860	*D80*	D87	ML62 *ML60* ML58 FL56 ML52
	M III:4	1866	1874	*U25*	D20	*ML60*
	M III:6	1869	1876	D79	*D28*	MD4 ML49 FD30
M III:3	M IV:9	1894	1902	*L60*	L52	*FL30* FL27
M III:4	M IV:13	1894	1907	*L60*	L47	*FL28 ML26*
M IV:9	M V:1	1910	1924	L44	*L30*	ML4 ML2 FL0
M IV:13	M V:3	1915	1926	D30	*L28*	*FL10*
	–,,–	1921	–,,–	L33	–,,–	*ML4*

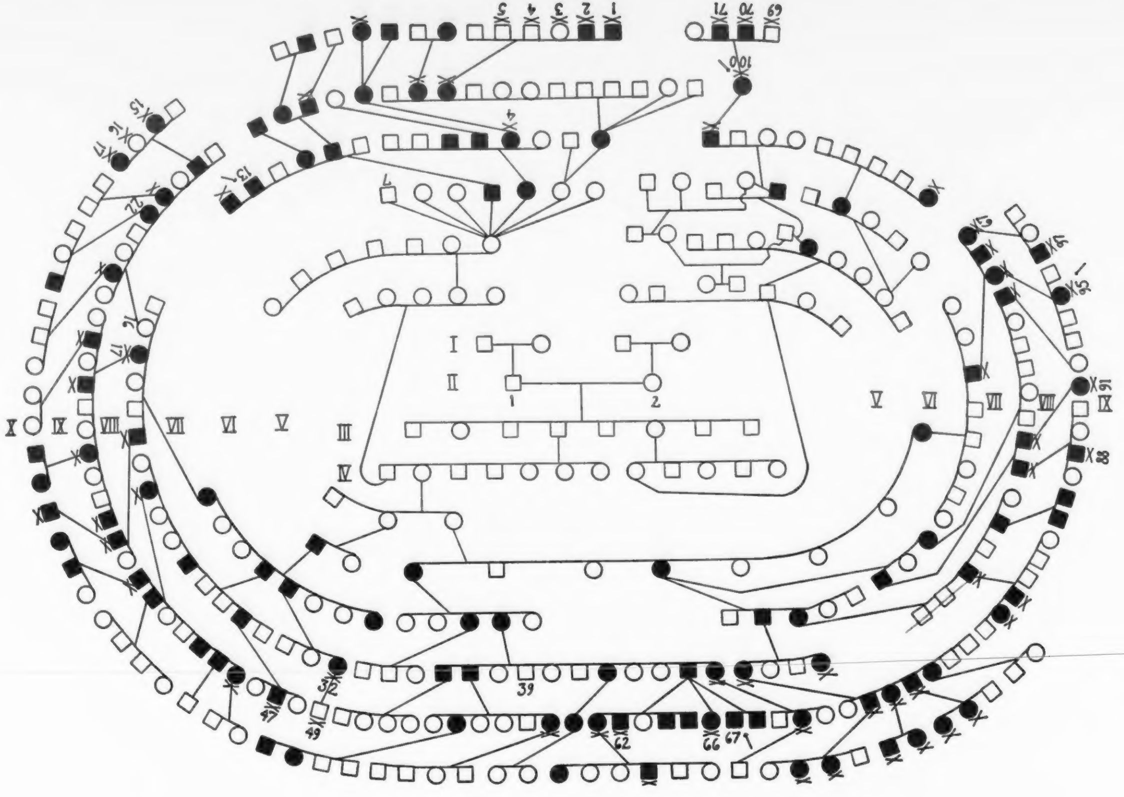

Fig. 10. Vånga family.

■ affected male
● affected female
○ female, unaffected or state of health unknown
↗ proband

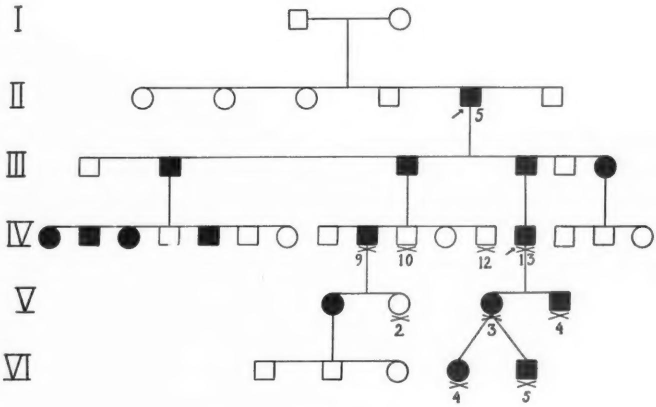

Fig. 11. Matteröd family.

■ affected male
● affected female
□ male, unaffected or state of health unknown
○ female, unaffected or state of health unknown
↗ proband
× examined personally

Table B. *Survey of clinical data*

1. Pedigree No. V = Vånga M = Matteröd.
2. Sex.
3. Source of information:
   P = personal examination
   L = by letter
   R = from relatives (01 = parent, 10 = child, 11 = sib, 20 = grandchild, 21 = nephew or niece, 22 = first cousin).
   M = Mjönes
   K = Kulneff
   D = the Danish colleagues Hauge, Helweg-Larsen & Sagild.
4. Age at and manner of disappearance from observation:
   L = living Dec 31, 1954
   D = dead
   U = untraceable
5. Type (see page 9).
6. Severity A = mild B = moderate C = severe.
7. Age at onset in years.
8. Frequency of attacks (schematic) S = several times a day D = once a day W = once a week M = once a month Y = once a year
9. Duration of attacks.
10. After 30 years of age. No improvement + Improvement —
11. Seasonal variation + No seasonal variation —
12. Nocturnal attacks + No nocturnal attacks —
13. After food intake. No improvement + Improvement —
14. During pregnancy. Deterioration + No deterioration —
15. Muscles innervated by cranial nerves: involved + not involved —
16. Respiratory troubles + No respiratory troubles —
17. Remarks

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
V VI:8	M	R20	D63													
V VI:10	F	R10	D76													
V VI:13	F	R20	D72													
V VI:17	F	R10	U54													
V VI:22	F	R20	U25													
V VII:3	F	R10	D67													
V VII:4	M	R20	D43													
V VII:8	F	R10	D86													
V VII:11	M	R20	D80													
V VII:12	M	R10	D68													
V VII:15	F	R21	D69													
V VII:18	F	R20	D60													
V VII:19	F	M	D92			7—9					+					
V VII:22	M	R10	D76													
V VII:23	F	R10	D38													
V VII:26	M	R10	D73													
V VII:28	F	R10	D64													
V VII:34	M	P	D71	2	B	1—4	D	12—24 hr	+	+	+	—		—	—	
V VII:40	F	R10	D81													
V VII:42	M	R10	D81													
V VIII:1	F	R22	D71													
[illegible]																
V VIII:6	M	R11	D22													
V VIII:10	M	D	L60			5—7	W	½—1 hr	—							
V VIII:11	F	D	D27													
[illegible]																

V VIII:6	M	R11	D22													
V VIII:10	M	D	L60			5—7	W	½—1 hr	—							
V VIII:11	F	D	D27													
V VIII:13	M	D	L52			7	S	1—2 hr	+	+	+	—		+	+	
V VIII:14	M	P	L49	2	B	7—9	D	½—1 hr	+	+	+	+		+	—	
V VIII:17	F	P	L71	1	B	7—9	W	½—1 hr	—	+	+	—	+	+	—	Atoxic goiter
V VIII:20	M	P	L60	2	C	7—9	W	24—72 hr	—	+	+	—		+	—	
V VIII:22	F	P	L67	2	B	1—2	M	½—1 hr	—	+	+	—	—	+	—	
V VIII:25	M	R11	D27													
V VIII:28	M	R10	D53									—				
V VIII:32	F	P	L57	1	A	7—9	M	½—1 hr			+	—	—	+	—	Hemicrania
V VIII:36	M	R11	D49													
V VIII:37	M	M	L75			18			+	+		—		—	—	
V VIII:42	F	R10	D42													
V VIII:45	M	M	L57	2	B	7—8	S	1—2 hr	+	+	+	—		—	—	
V VIII:46	F	P	L55	1	B	8—9	W	1—2 hr	+	+	+	+	—	—	—	
V VIII:47	F	P	L64	2	B	7—9	M	½—1 hr	—	—	+		—	+	—	
V VIII:50	F	P	L46	2	C	7—9	D	15—60 min	+	+	+			+	—	
V VIII:53	M	P	L57	1	A	2—4	M	10—15 min		—	—	+		—	—	
V VIII:55	M	L	L53	2	B	9			—	+	+			+	—	
V VIII:57	M	P	L44	2	B	1—4	M	10—30 min	+	+	+	—		—	—	
V VIII:58	M	P	L38	2	C	1—4	D	12—24 hr	+	+	+	—		+	—	
V VIII:64	M	P	L36	2	B	5—7	D	½—1 hr	+	+	+	+		—	—	
V VIII:65	F	P	L45	1	B	1—4	W	½—1 hr	—	—	—	+	—	—	—	
V VIII:66	M	P	L43	2	B	1—4	D	1—2 hr	+	+	—	+		—	—	
V VIII:67	F	P	L41	2	A	31	W	15—30 min		—	—		+	—	—	Attacks only during pregnancies
V VIII:68	F	P	L64	1	B	5—7	W	15—20 min	—	—	+	+		—	—	
V VIII:76	M	P	L61		B	9	W	5—6 hr	—	+	+	+		+	+	
V IX:10	F	P	L38	1	B	7—9	M	½—1 hr	—	+	+	+	+	—	—	
V IX:11	F	P	L34	1	B	7—9	W	½—1 hr	+	—	+	+	+	+	—	
V IX:13	F	R01	D23			7				+			—			
V IX:15	M	P	L30	1	A	18—19	Y	5—10 min		—	+	—		—	—	
V IX:16	F	D	L29			1—4	S	15—30 min		+	+	—	+	—	—	
V IX:17	M	D	L32			5—7	S	½—1 hr	+	+	+	—		+	—	
V IX:19	M	R10	D48													
V IX:21	F	P	L49	1	A	7	M	½—1 hr		—	+	—	—	+	—	
V IX:22	F	L	L47			5—9			—	—	+	—	+	+	—	Op. toxic goiter 1941
V IX:25	F	P	L38	1	A	13—14	Y	5—10 min		—	+	+	+	—	—	
V IX:28	M	P	L41	1	A	7—9	M	½—1 hr		+	+	—		+	—	
V IX:30	M	P	L37	1	B	5—7	S	½—1 hr	+	+	—	—		+	—	
V IX:33	F	P	L32	1	B	13—14	D	½—1 hr	—	+	—	—	+	+	—	

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
V IX:36	M	P	L26	1	B	11	S	$\frac{1}{2}$—1 hr		+	+	—		+	+	
V IX:37	M	P	L34	2	B	7	M	2—3 hr	—	+	+	—		+	—	
V IX:39	M	P	L45	2	B	1—2	W	$\frac{1}{2}$—1 hr	+	+	+	—		+	—	
V IX:40	M	R01	L43		C	1—2	D	1—2 hr			+					
V IX:43	M	R01	L38		B	1—2	W	$\frac{1}{2}$—1 hr		+		—				
V IX:44	M	R01	L34		A	1—2	Y	10—20 min			—	—				
V IX:45	F	P	L32	2	B	7—9	W	$\frac{1}{2}$—2 hr	+	+	+	—	—	+	—	
V IX:47	M	P	L20	2	C	5—6	W	24—72 hr		—	+	—		+	+	Hospital case No. 13
V IX:55	F	M	L45	1	A	8—9	Y	$\frac{1}{2}$—1 hr		—	—		—	—	—	
V IX:59	F	P	L36			15	D	1—2 hr	—	—	—		—	—	—	
V IX:60	F	M	L40		B	5—7	W	$\frac{1}{2}$—1 hr	+	+	+		—	—	—	
V IX:61	F	P	L32	1	B	3—4	W	$\frac{1}{2}$—1 hr	—	+	—	—	+	—	—	
V IX:62	M	P	L31	2	A	7—9	W	20—30 min		+	—	+		—	—	Hospital case No. 17
V IX:64	M	M	L26	1	B	7—9	M	$\frac{1}{2}$—1 hr		+	—	—		—	—	
V IX:65	M	M	L23	1	B	3—4	S	1—2 hr		—	+			—	—	
V IX:66	F	P	L20	1	B	4	W	$\frac{1}{2}$—1 hr		+	+	—	+	—	—	Hospital case No. 15
V IX:67	M	M	L17	1	B	$2\frac{1}{2}$	S	$\frac{1}{2}$—1 hr		—	—	—		—	—	
V IX:68	M	M	L14			2—3										
V IX:70	F	P	L32	1	B	6	S	15—30 min	+	+	+	+	—	+	+	
V IX:73	M	P	L42	2	B	7	Y	$\frac{1}{2}$—2 hr	—	+	+	—		—	+	
V IX:74	F	P	L40	2	B	7—9	W	15—20 min	—	—	—	—	—	+	—	
V IX:75	M	P	L34		B	5—7	Y	$\frac{1}{2}$—1 hr	+	—	+	+		+	—	
V IX:76	F	L	L32	1	A	12—13	Y	15—20 min		—	—	—	—	—	—	
V IX:80	F	P	L21	1	A	2—3	W	5—10 min		—	+	+		—	—	
V IX:81	M	P	L19	1	B	12—14	M	20—30 min		+	—			—	—	
V IX:85	M	M	L24	2	B	5	D	1—2 hr		+	+			+	—	
V IX:86	M	M	L21		B	2—3	W	1—2 hr			+	+		+	—	
V IX:88	M	P	L11	2	B	1—4	M	$\frac{1}{2}$—1 hr		+	+	—		+	—	Hospital case No. 2
V IX:91	F	P	L11	2	B	$1\frac{1}{2}$	D	$\frac{1}{2}$—1 hr		+	+	—		+	—	Hospital case No. 3
V IX:95	F	P	L16	1	B	2—3	W	1—2 hr		—	—	+		+	—	Hospital case No. 1
V IX:97	M	P	L20	2	B	7—9	W	2—4 hr		+	—	—		—	—	Hospital case No. 7
V IX:100	F	P	L37	2	B	2—3	M	$\frac{1}{2}$—1 hr	—	+	+	—	+	+	+	Hospital case No. 16
V X:1	M	P	L16	1	A	3	D	$\frac{1}{2}$—1 hr		—	+	+		—	—	Hospital case No. 11
V X:2	M	P	L14	1	A	7	M	15—20 min		—	+	+		—	—	Examined during attack
V X:7	F	R01	L14	1	A	9	M	$\frac{1}{2}$—1hr		+	—	+		—	—	
V X:9	M	L	L18	2	B	3—4				+	+	—		+		
V X:10	F	P	L13	2	B	$1\frac{1}{2}$—2	W	10—30 min		+	+	—		+	—	
V X:12	M	D	L5			$1\frac{1}{2}$	S	15 min		+	+	—		—	—	

V X:22	M	L	L6			[illegible]										
V X:28	M	R01	L9	1	B	2—3	D	½—1 hr		+	+	—		—	—	
V X:29	F	R01	L7	1	B	5	D	½—1 hr		+	—			—	—	
V X:30	M	P	L10	1	B	2	W	½—1 hr		+	—	—		—	—	
V X:31	F	R01	L14	1	B	7	S	5—15 min		+	+	—				
V X:32	M	R01	L10	1	B	5	S	5—15 min		+	+	—				
V X:41	M	M	L18	2	B	5	D	1—2 hr		—	+			—	—	
V X:42	F	M	L14	1	B	2—3	D	2—4 hr		—	—			—	—	
V X:51	F	R01	L12	1	A	7—9	M	10—15 min		—	—	—		—	—	
V X:54	M	P	L7	1	A	6—7	M	5—10 min		+	—	+		—	—	
V X:59	F	P	L13	2	B	5	M	15—20 min		—	+	—		—	—	
V X:60	F	P	L7	2	B	5	W	5—15 min		—	—	—		—	—	
V X:62	M	P	L17	1	B	7—9	W	15—20 min		+	+	—		—	—	
V X:63	F	P	L12	1	B	7	D	15—20 min		—	+	—		+	—	
V X:64	F	P	L13	1	B	7—8	W	½—1 hr		—	+	—		—	—	
V X:65	F	P	L9	1	B	6—7	D	½—1 hr		—	—	—		—	—	
V X:70	M	P	L10	1	B	3—4	D	30—40 min		+	+	—		+	—	Hospital case No. 8
V X:71	M	P	L5	1	A	4	W	10—20 min			—			—	—	Hospital case No. 9
M II:5	M	K	D59													
M III:2	M	K	U50			0—4					+					
M III:3	M	K	D80			0—4				+	+					
M III:4	M	K	U25								+					
M III:6	F	K	D28													
M IV:1	F	K	D16			0—4										
M IV:2	M	K	D20			1—2										
M IV:3	F	K	D3			1—2										
M IV:5	M	K	U14			0—2										
M IV:9	M	P	L60	1	B	1—2	W	<1 hr	—	+	+	+		—	—	Hospital case No. 12 Slight permanent weakness of the arms
M IV:13	M	P	L60	2	C	2—4	W	4—8 hr	+	+	+	+		+	—	Hospital case No. 10 Permanent weakness and atrophy of the muscles of the arms and legs for about 10 years
M V:1	F	L	L30		B	1	D	15—30 min	+	+	+	+	+	—	—	
M V:3	F	P	L28	2	B	1	D	1—2 hr		+	+	+	—	+	—	Hospital case No. 6
M V:4	M	P	L26	2	B	3—4	W	2—4 hr		+	+	—		+	—	Hospital case No. 14
M VI:4	F	P	L10	1	B	1	S	5—30 min		+	+	+		+	—	Hospital case No. 4
M VI:5	M	P	L4	1	B	8/12	S	10—30 min		+	+	+		—	—	Hospital case No. 5

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